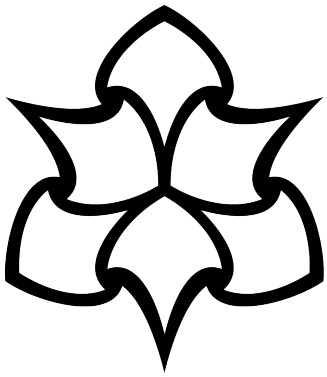

POTENTIALLY TOXIC ELEMENTS AND PERSISTENT ORGANIC POLLUTIONS IN HAIR: A CASE STUDY OF GREATER MANCHESTER

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A thesis is submitted in partial fulfilment of the requirements of the Manchester Metropolitan University for the degree of Master of Science (by Research)

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Declaration

I declare that the thesis is submitted by me for the Master of Science degree, and is solely my own research in the Department of Natural Sciences, Manchester Metropolitan University. All of the support received from other individuals has been acknowledged, and full reference is made to the published sources used. Prior to my submission, this thesis has not been submitted either by me or other universities. I also give up copyright of the dissertation in support of Manchester Metropolitan University.

Hülya Kars

October 2018

Abstract

This study aimed to compare metal and Persistent Organic Pollutant (POP) concentrations in human hair clippings between metropolitan boroughs in Greater Manchester with different death rates.

The official statistics for death rates for the years 2014-2017 and life expectancy data were used to identify nine boroughs in Greater Manchester for the study. Five of these are located predominantly in the northern and western parts of the city and reports the highest death rates. These boroughs were Bolton, Bury, Rochdale, Oldham and Wigan. Four other boroughs mainly in the central and southern parts of the city with low annual death rates were also identified for use in the work, namely Manchester, Trafford, Stockport and Salford. In each borough, two male and two female hairdresser shops were chosen for the sample collection, and hair of each type were combined to form in a composite hair sample through the application of the quarter and coning technique.

Different hair washing methods were evaluated to determine their effect on the metal analyses of each hair sample, and the results were then compared with the published literature. For the remainder of the work reported in this study, a simple wash (deionised water wash) was selected as the sample preparation method. The metal contents were extracted by means of microwave digestion and analysed by ICP-OES. QUeChERS combined with a dispersive solid phase extraction method, was used to extract the POPs (i.e. polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)) and Gas Chromatography-Mass Spectroscopy was employed for the analysis.

The focus was primarily on the contents of Cd, Pb, Ni, Cu, Zn and Mn analyses as far as the metallic elements were concerned, due to their toxic and carcinogenic behaviour in the human body. Various typical PCBs and PAHs were analysed in the POPs determinations. It was found that Pb and Zn were the two metallic elements with the highest concentrations in both the male and female hair samples in each borough from which hair samples were collected. For the PCB analyses, PCB 118 was present in much higher concentrations than

the rest in both the female and male hair samples, and was also present to a much larger extent in the female hair samples than in the male samples. The three PCBs, apart from PCB118, had similar concentrations compared to each other in both the male and female hair samples.

During the PAHs measurements, the analysis revealed that the PAH contents are dominated by these three compounds, i.e. acenaphthene, anthracene and naphthalene. In the female hair samples high values of acenaphthene were found. In the male hair samples, acenaphthene was also found in higher concentrations than any of the other PAHs, while naphthalene occurred in higher concentrations in the male hair samples than the rest of the PAHs, but was generally lower in concentration than acenaphthene.

There was no discernible correlation between the death rates and/or life expectancy of the population in the various boroughs of Greater Manchester and the metallic element concentration profiles and POPs contents of the male and female hair samples.

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Abbreviations

AA	Atomic Absorption
ACN	Acetonitrile
ATSDR	Agency for Toxic Substances and Disease Registry
CHMP	Committee for Human Medicinal Products
CIEL	Centre for international Environmental Law
COPD	Chronic Obstructive Pulmonary Disease
DDT	DICHLORODIPHENYLTRICHLOROETHANE
DIW	Deionized Water
dSPE	dispersive Solid Phase Extraction
GC	Gas Chromatograph
GC-MS	Gas Chromatography-Mass Spectrometry
GHO	The Global Health Observatory
GM	Greater Manchester
GMHSCP	Greater Manchester Health and Social Care Partnership
HCB	Hexachlorobenzene
HMs	Heavy Metals
HNO_3	Nitric acid
HPLC	High Performance Liquid Chromatography
IAEA	International Atomic Energy Agency
ICP-MS	Inductively Coupled Plasma-Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer
IHD	Ischaemic heart disease
IQ	Intelligence Quotient
LOD	Limit of Detection
LOQ	Limit of Quantification
MAD	Microwave-assisted Acid Digestion
mg kg^{-1}	Milligram per kilogram
µg g^{-1}	Microgram per gram
MMU	Manchester Metropolitan University

MSD	Mass Selective Detector
NHS	National Health Services
ng g^{-1}	Nanogram per gram
ONS	Office for National Statistics
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCDD	Polychlorinated dibenzo-p-dioxins
PCDF	Polychlorinated dibenzofurans
pg μL^{-1}	Picogram per microlitre
POPs	Persistent Organic Pollutants
ppb	Parts per billion
ppm	Parts per million
PTFE	Polytetrafluoroethylene
SCCPs	Short-chain chlorinated paraffins
SEM	Scanning Electron Microscopy
UK	United Kingdom
WAW	Water-Acetone-Water
WHO	World Health Organisation

1 INTRODUCTION

1.1 Background

Life expectancy is a clear sign of population health and exhibits the general mortality level of the population (Waldram et al., 2006). By definition, life expectancy is the average number of years that a human being is expected to live and is based on current death rates (Carter and Slack, 2010:112). The Global Health Observatory (GHO) data provide the world life expectancy at birth in 2015 as 73.8 years for females, and 69.1 for males, with a reported difference between the sexes of 4.7 years (World Health Organisation, 2017). A similar trend is seen in the United Kingdom (UK), where the Statistical Bulletin National life tables 2014 to 2016 reveal that the life expectancy at birth is 82.9 years for females and 79.2 years for males (Sanders, 2017).

The focus of this research is on the life expectancy in specific areas of Greater Manchester (GM), and whether hair analysis may be correlated to this. The total death rates in GM were reported to be 21,700, 22,548, 22,236 and 22,512 in 2014, 2015, 2016 and 2017, respectively (Patel, 2017). Purdam (2016) highlights that life expectancy varies in GM, according to areas. For example, the estimated life expectancy in Timperley village (in the borough of Trafford) is 78.3 years for men and 81.3 years for women, while in Rochdale it is only 65.7 and 74.3 years, respectively. Male and female death rates and the difference between the sexes within the GM counties are shown in Figures 1.1–1.3. Wigan, Trafford, Stockport, Salford, Bury had a higher female than male death rate in 2016 and 2017. While the male death rate was higher than the female one in Manchester in 2014 and 2016, in 2015, the death rate was nearly the same for both sexes (0.7%). The male death rate was higher than the female one in 2014 and 2017 in Rochdale, and in 2014 in Wigan. This, however, changed in the subsequent years. Rochdale had fewer male deaths in 2015, while Wigan had more female deaths in 2016.

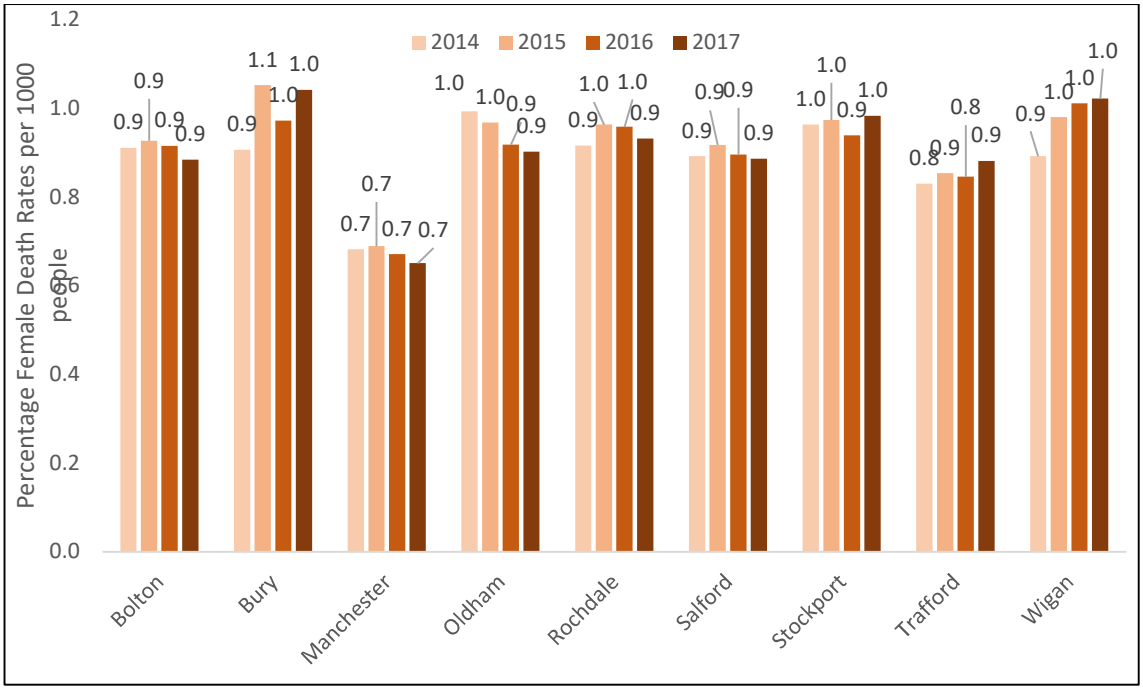

Fig.1.1 :Percentage of female deaths rates per 1000 people for all ages by area of usual residence in GM from 2014–2017. (Data obtained from Patel, 2017.) The figures above the bars indicate the % values.

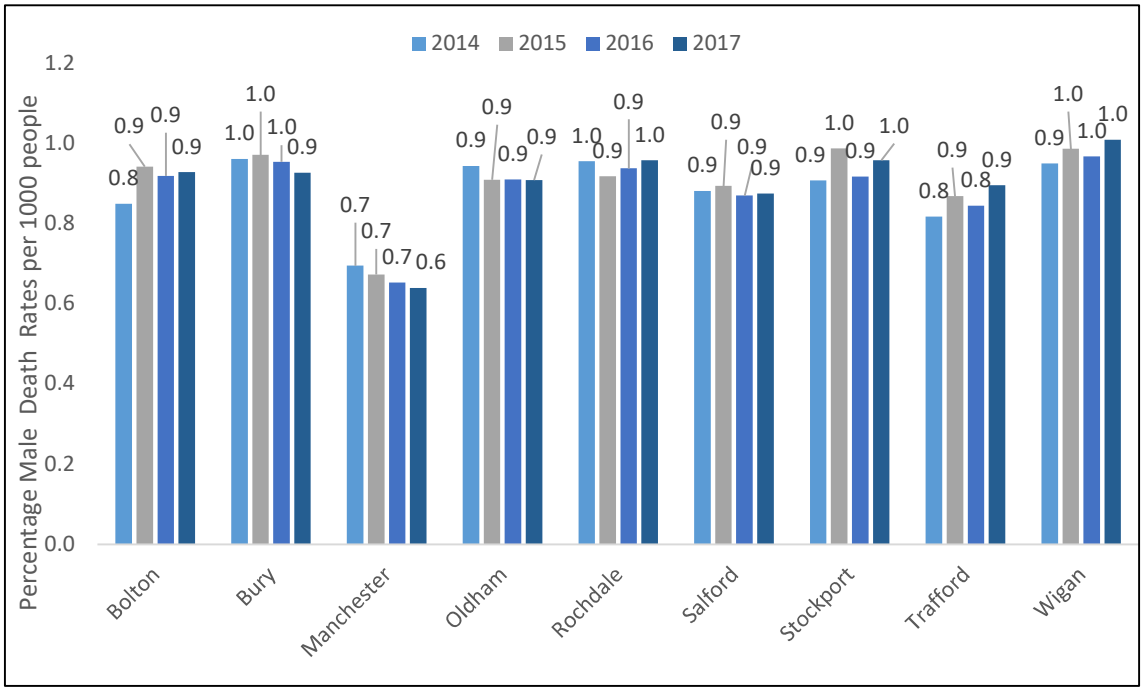

Fig.1.2 :Percentage of male deaths rates per 1000 people for all ages by area of usual residence in GM from 2014–2017. (Data obtained from Patel, 2017.)

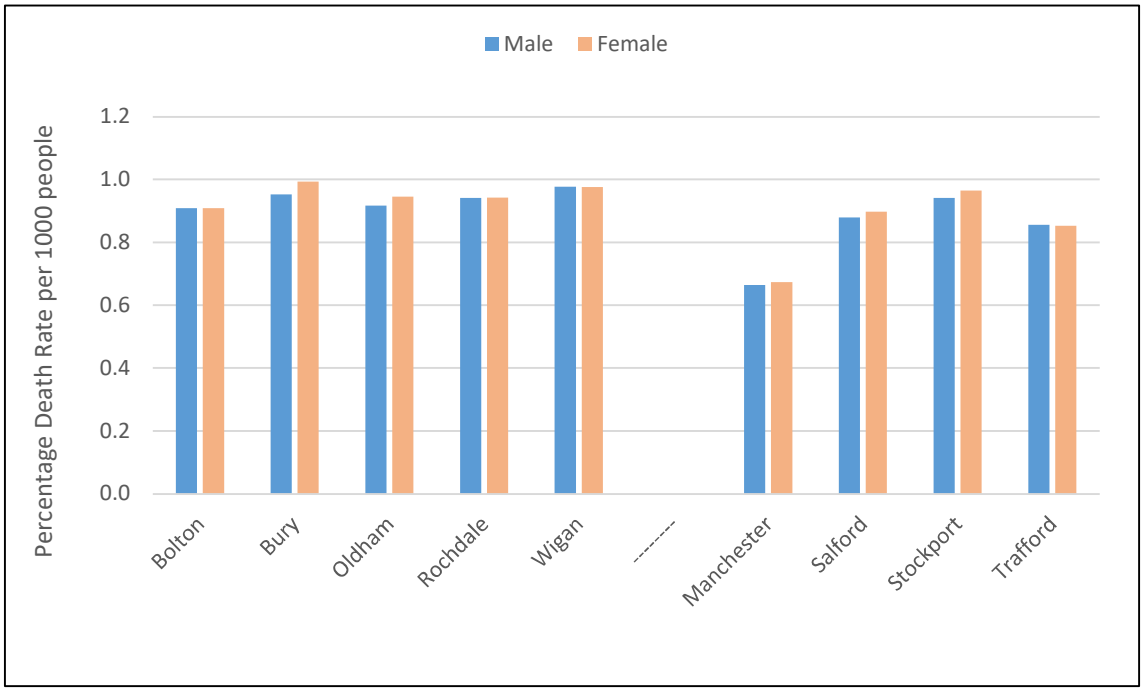

Fig.1.3 :Percentage of Male vs Female death rates per 1000 people for all ages by area of usual residence in GM from 2014–2017. (Data obtained from Patel, 2017)

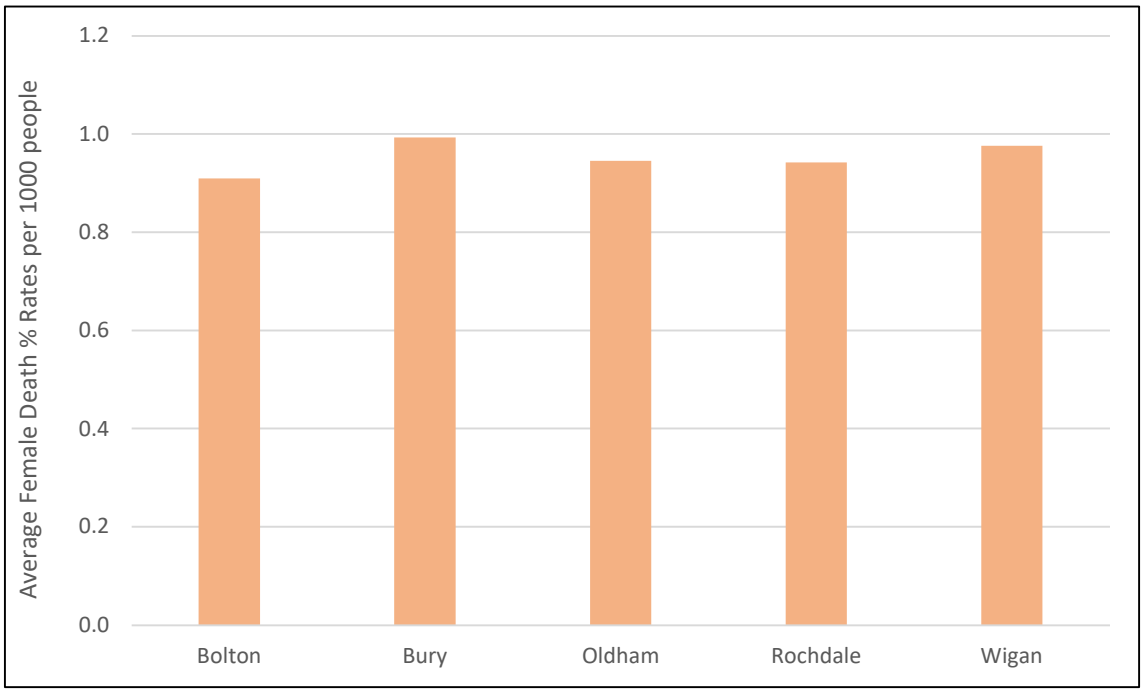

Fig.1.4 :The higher female mortality areas in northern GM from 2014–2017. (Data obtained from Patel, 2017.)

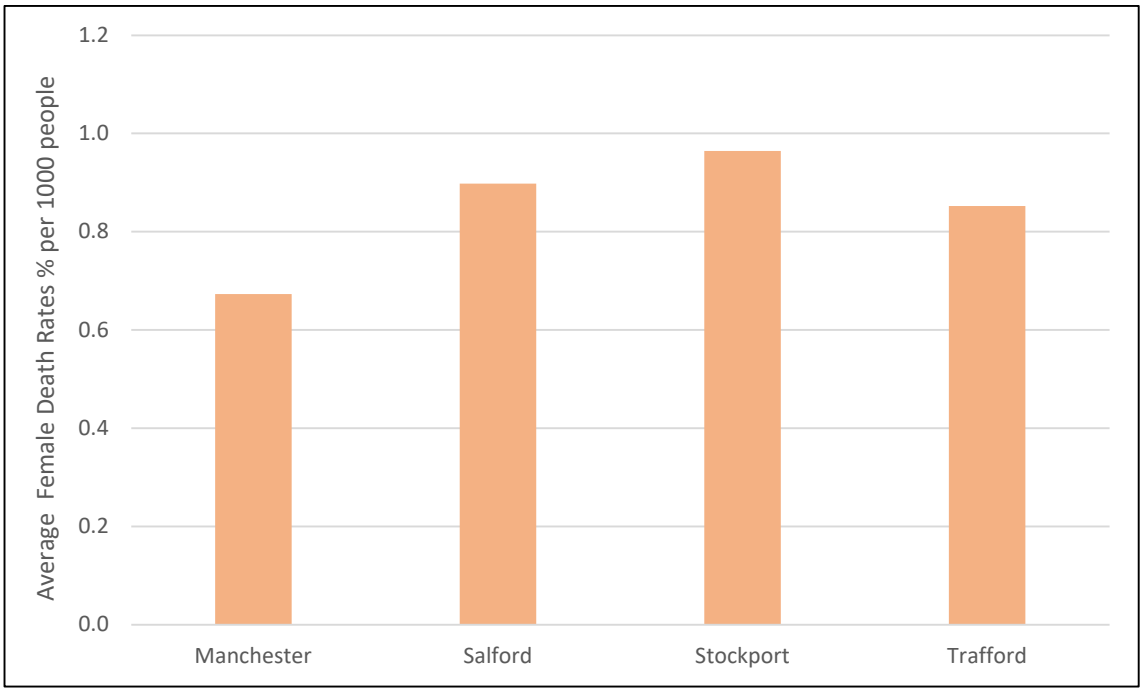

Fig.1.5 :The lower female mortality rate areas in southern GM from 2014–2017. (Data obtained from Patel, 2017.)

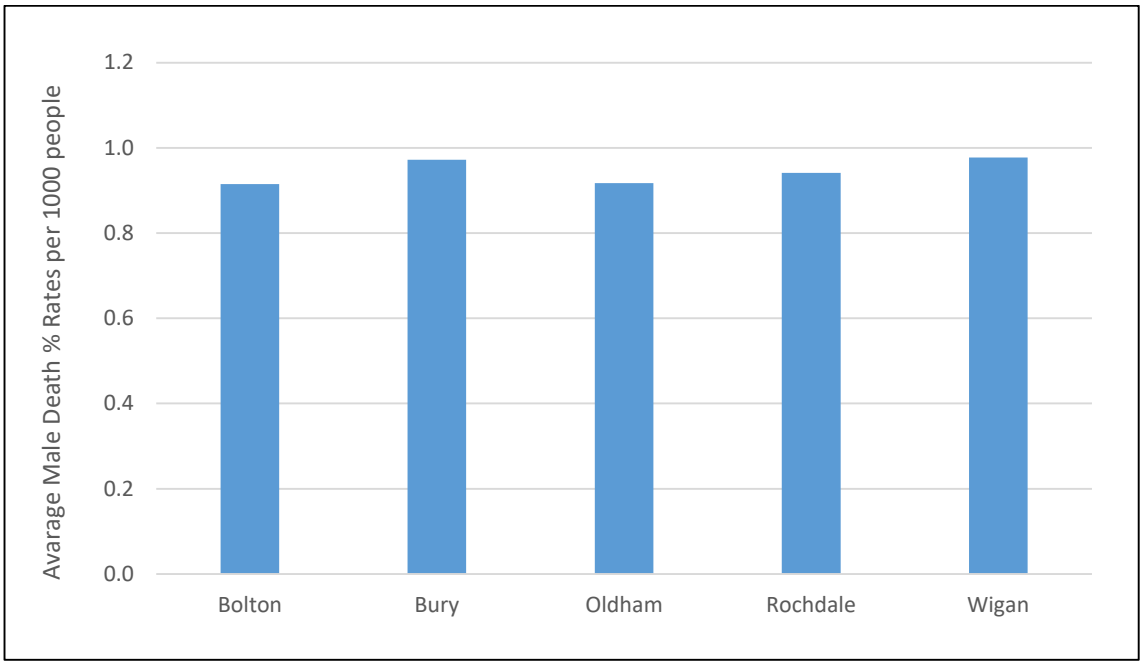

Fig.1.6 :The higher male mortality areas in northern GM from 2014–2017. (Data obtained from Patel, 2017.)

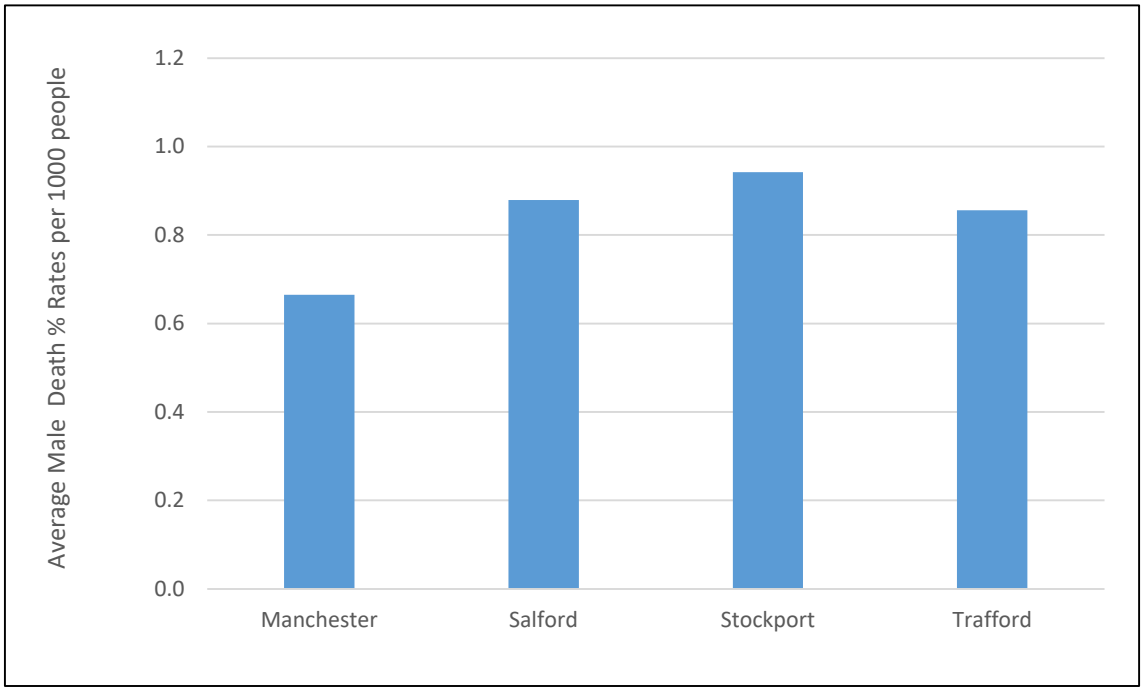

Fig.1.7 :The lower male mortality areas in southern GM from 2014–2017. (Data obtained from Patel, 2017.)

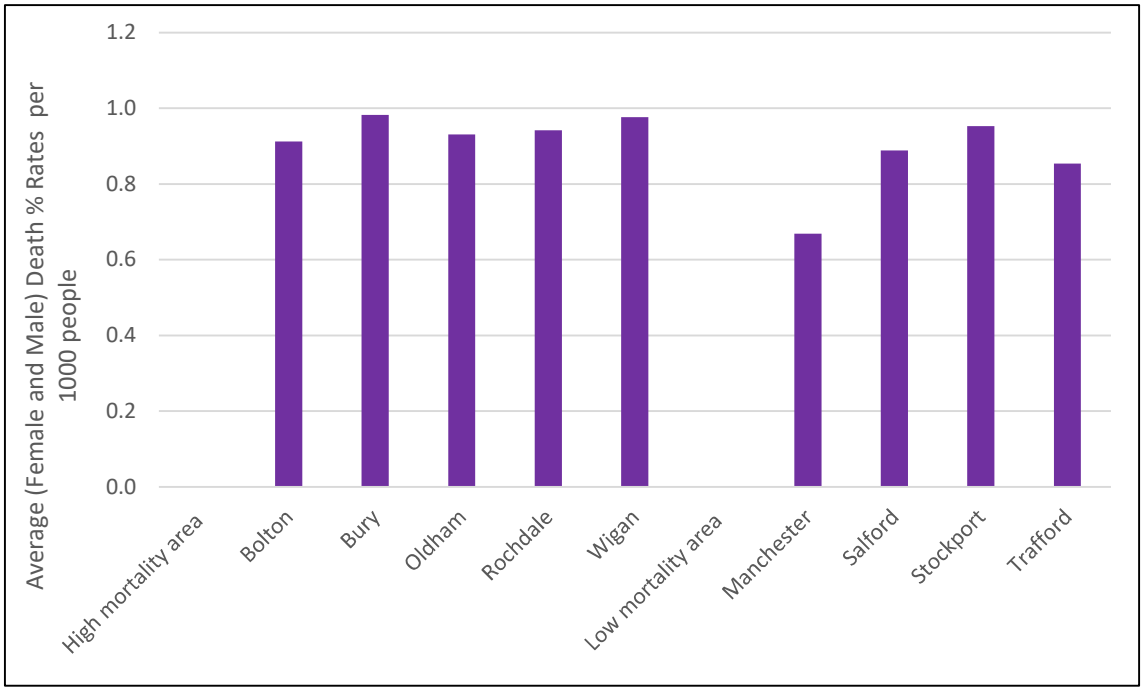

Fig.1.8 :The higher and lower mortality areas in GM from 2014–2017. (Data obtained from Patel, 2017.)

The standardised accumulative death percentage rates worked out for Bury, Oldham, Rochdale, Stockport, Wigan and Bolton were within experimental error and remained stable throughout the four-year periods under consideration. Bolton, Bury, Oldham, Rochdale and Wigan had higher mortality rates than Manchester, Salford, Stockport and Trafford. In the boroughs of Bury, Manchester, Salford, Stockport, Trafford and Wigan, the female mortality rate percentage in 2016 and 2017 were higher than those of the males. Overall, Manchester had a relatively lower death rate percentage for both males and females for all the areas of the city considered, as shown in Figs.1.1–1.3. The highest male death rates were recorded in Stockport and Wigan in 2015. Overall the mortality rates were rated 1 to 9 using the data, as well as dividing the boroughs as northern and southern areas (Table 1).

Table 1 :Mortality Rate (highest to lowest) within the GM regions for 2014–2017

Highest (1-5)					**Lowest (6-9)**			
1	2	3	4	5	6	7	8	9
Bury	Wigan	Rochdale	Oldham	Bolton	Stockport	Salford	Trafford	Manchester
Note: 1 is the highest and 9 the lowest. Based on the standardised total death rates per 1000 people over a four-year period. (Raw data gained from Patel, 2017a.)								

From 2009 to 2011, the mortality rate at age 65 in the UK decreased due to the reduced mortality from circulatory diseases, motivated by changing smoking habits, the improved diagnosis and treatment of cancer, as well as technological and medical advances in treating other diseases and syndromes (Murphy and Di Cesare, 2012; Sanders, 2017). Despite these improvements, the broad disease categories of cancer (neoplasm), circulatory disease and respiratory disease made the biggest contribution to deaths in 2013 (McLaren, 2014). The Office for National Statistics (ONS, 2017) reported that the five top leading causes of death in the Manchester area from April 2015 to March 2016 were ischaemic heart disease, malignant neoplasm of the trachea bronchus in the lungs, chronic lower respiratory disease, dementia and Alzheimer's disease, influenza, pneumonia and Cerebrovascular disease.

From 2011 onwards, Government cuts had a noticeable adverse effect on people's life expectancy in the UK (Slay and Penny, 2013; Poinasamy, 2013; Wintour, 2013). The resulting austerity was linked to decreases in welfare spending, disability benefits and health services, such as the NHS and adult social care, which affected life expectancy in the UK in 2017 and increased the mortality rates, particularly among the older population (Dorling and Gietel-Basten, 2017). '*The environment can shape people's behaviour and limit or enhance their wellbeing and life changes*' and this is not understood by policies (Pinoncely, 2016:1). Marmot (2010) reported that the inhabitants of the richest districts in England live longer than those of the poorest districts, as health inequality exists in society. Simple socio-economics, epigenetics, living standards, lifestyle, working standards and educational factors determined the mortality rates in northern UK (Mitchell et al., 2000; Hacking et al., 2011; Bennett et al., 2015; Lupton et al., 2015).

Human life expectancy is affected not only by lifestyle/diet, but also by environmental factors such as air and land quality. The Environmental Protection Act 1990 and Environment Act 1995 hold each borough council responsible for inspecting their area and monitoring the air quality and land contamination. This becomes even more important when the local authorities are considering using their land resources for new developments within the built-upon areas.

1.2 Organic Pollutants

There is a growing body of literature that recognises the importance of Persistent Organic Pollutants (POPs), Polycyclic Aromatic Hydrocarbons (PAH) and Polychlorinated Biphenyls (PCBs) and toxic metal elements, that have an adverse effect on human health, and which are relevant to life expectancy and mortality rate.

1.2.1 Persistent Organic Pollutants (POPs)

POPs are toxic to wildlife and humans, even at relatively low concentrations (ATSDR, 1995; Robertson et al., 2004; Environmental Protection Agency, 2009). Carbon based organic chemical substances of POPs emissions leave persistent and specific physical and chemical footprints in the environment and are distributed through the soil, water and air. Carpenter (1998) stated that POP's accumulate in the fatty tissues of humans and other living organisms, as they are not soluble in the water. They seriously disrupt immune systems (Gascon et al., 2013), alter thyroid functions (Schell et al., 2008) and hormonal systems (Arcaro et al., 1999), damage central nervous, and reproductive systems, have developmental (Kodavanti, 2005) and carcinogenic (Carpenter and Bushkin-Bedient, 2013) outcomes, cause ischemic heart disease (Burstyn et al., 2005), lead to hypersensitivity and allergies (Tsuji, 2015), and are a risk factor for cardiopulmonary and lung cancer mortality (Pope et al., 2002).

From the industrial revolution onwards, Manchester has experienced mass production, achieved by new machinery and technology. The cotton and metalwork industries have become internationally important to the economy, and have brought about a fundamental change to the nature of British life (British Museum, 2017). The result is that massive quantities of hydrocarbons are contaminating the environment; for instance, PAHs. Over time, land/soil contamination has occurred through spillages or accidental debris as a result of land use; for example, waste yards, transports, gasworks, power generation, chemical industries, plants, etc., especially when these areas are close to/within residential areas (Ashworth et al., 2005). Certain POPs occur naturally, like the PAHs, which are mainly produced in the open air through the incomplete combustion of coal, oil, petrol or wood. Many of the PAHs have toxic, carcinogenic and mutagenic characteristics (Abdel-Shafy and Mansour, 2016). PAHs do not appear as single compounds, but usually as complex mixtures; for instance, soot. Pure single compounds are manufactured for research purposes. Furthermore, some of the PAHs are used to prepare pesticides, plastic and dyes, as well as for medicine (ATSDR, 1995). The chemical structures of several PAHs are illustrated in Fig.1.9.

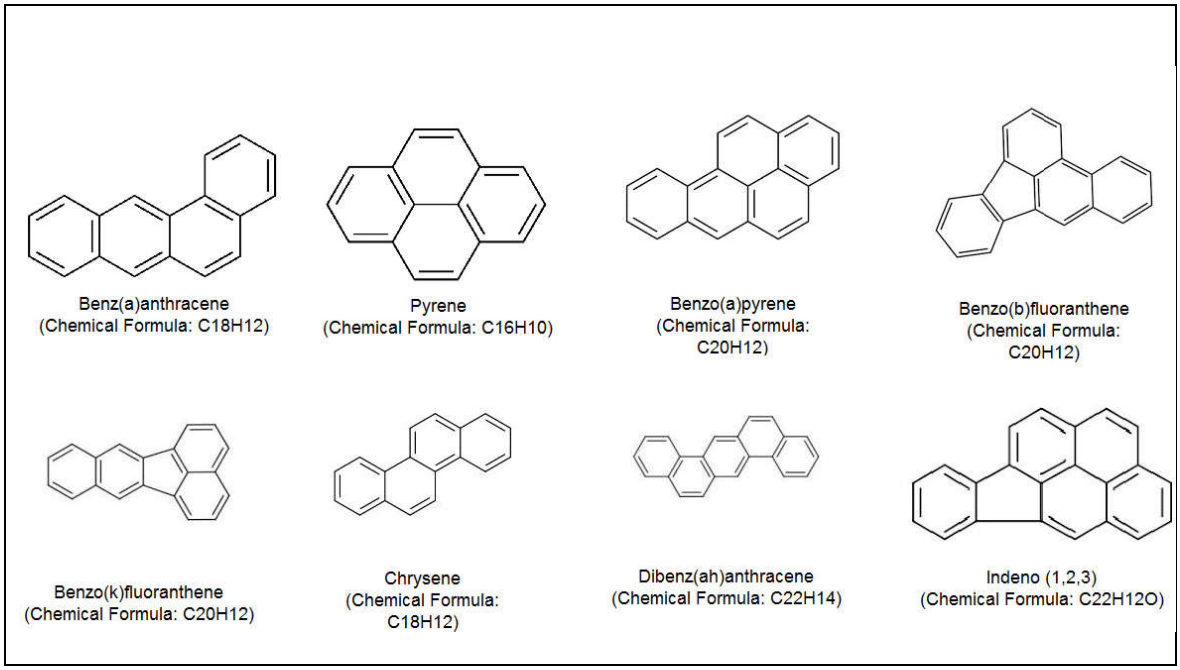

Fig.1.9 :Chemical structure of PAHs' compounds (Poly Aromatic Hydrocarbons).

1.2.2 Polychlorinated biphenyls (PCBs)

PCBs are defined as industrial, synthetic, and organochlorine chemicals (United Nation Environment, 2017) and their use is not permitted. PCBs are made from the biphenyl molecule, which consists of 12 carbon atoms with chlorine atoms substituted for hydrogen atoms in any of the ten possible positions. A total of 209 dissimilar PCB components (congeners) are known, and named based on the number of chlorines and their position on the biphenyl rings. PCBs are fat-soluble substances, exposure to which can occur through the consumption of animal fats, breathing and/or skin contact (Carpenter, 2006). The volatile lower-chlorinated PCB components are metabolised without difficulty by the human body, as reported by Liebl et al. (2004:315). One of the main dangers of PCBs is that they persist in the environment and biological systems (Carpenter, 2006).

Exposure to PCBs endangers and weakens the immune system, which jeopardises human health and raises the risk of getting numerous illnesses, such as ischemic heart disease, cardiovascular and liver disease, diabetes, asthma and arthritis (Carpenter, 1998 and 2006). Some of the PAHs' and PCBs' adverse health effects are irreversible, such as decreasing

Intelligence Quotient (IQ) (Jacobson and Jacobson, 1996). The chemical structure of the PCBs are provided in Fig.1.10.

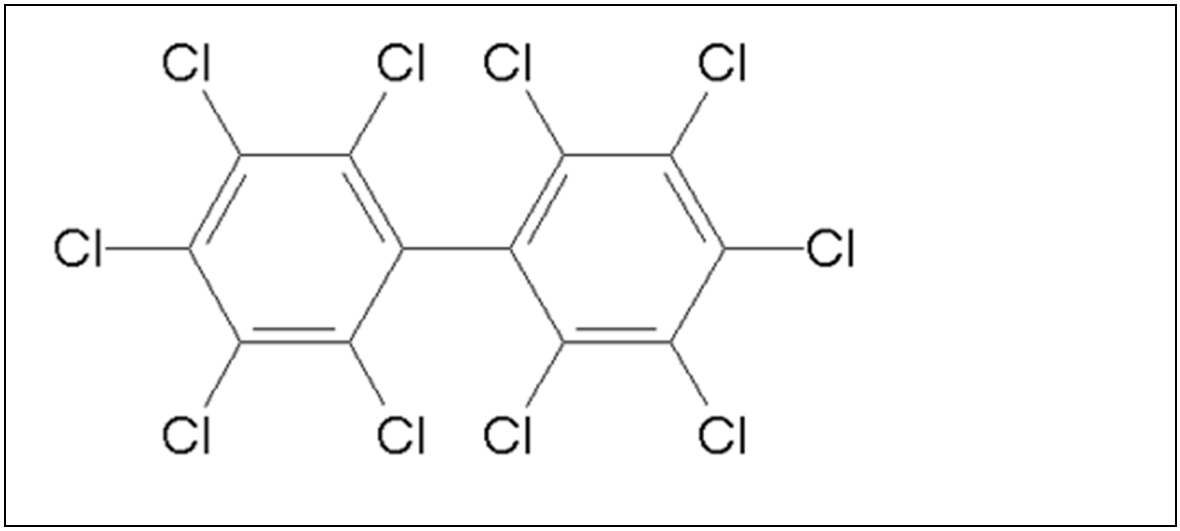

Fig.1.10 :Chemical Structure of PCBs' compounds (Polychlorinated biphenyls).

1.2.3 Heavy Metals (HMs)

Not only are organic molecules such as POPs detrimental to one's health, but some metals/metallic elements contribute adversely to human health as well. About two thirds of the elements in the periodic table are metals. Metals typically have high electrical and thermal conductivity, and reflectivity (metallic luster) (Klaassen, 2008). Moreover, when metals occur as ions in aqueous solutions some of them can display toxic effects (Smith and Nordberg, 2015).

Some metals act as nutrients to the human body. Calcium, cobalt, chromium, copper, iron, magnesium, manganese, potassium, sodium and zinc are all essential elements for humans. Calcium, zinc and magnesium are particularly useful for controlling the human body mass index (Yasuda et.al., 2006). There is a link reported between BMI, obesity and cancer in the UK (Hooper et al., 2018), and particularly obesity is linked to 6% of cancer cases in the UK (Brown et al., 2018). Similarly, manganese plays a role in regulating normal brain functioning (Crosby, 1998).

A reduced concentration of essential metals in the human body, such as chromium (III), iron, zinc and manganese, threatens human health (IARC, 2012; Mehra and Thakur, 2016; Savabieasfahani et al., 2012; Nordberg et al., 2015). Zinc is required for growth and development, mainly of the brain. On the other hand, excessive zinc levels negatively affect the copper balance in the body, as observed in Wilson Disease patients. A small amount of even 20mg Fe/kg is enough to generate gastrointestinal symptoms (Committee for Human Medicinal Products (CHMP), 2007:29). The US Department of Health and Services (2011:1) recommends a daily zinc intake for age 1–3 of 3mg, for age 4–8 of about 5mg, for age 9–3 of around 8mg, and for age 14–18 years 11mg for boys and 9mg for girls while, for adults, it is 11mg for males and 8mg for females.

A deficiency or excess of metal elements is detrimental to the role of certain organs (Ambiga, 2017). For example, iron deficiency has been noted in patients with Parkinson's Disease (Forte et al., 2005). The presence of unnecessary metals, which serve no purpose in the body, such as chromium (IV), lead, cadmium and nickel, adversely affects human health (IARC, 2012; Mehra and Thakur, 2016; Nordberg et al., 2015). Aluminium, arsenic, cadmium and lead are toxic metals, known for their interference in standard biochemical roles and as affecting various balances in the body (Rao, 2005). Nickel, being a moderately toxic element, can also cause respiratory disorders and cancer when inhaled (Gollhausen and Ring, 1991; Ferreira et al., 2001). All elements at elevated levels may be life-threatening and can cause a deficiency in certain nutrients.

Several studies illustrate that a toxic level of metals, as well as an excess/deficiency of essential metals, play a significant role in pathogenesis and negatively affect human health in the long term (Geier et al., 2009; Trojsi et al., 2013; Sas-Nowosielska and Pawlas, 2015). The chemicals aluminium, cadmium, lead, PCBs, arsenic, and toluene, and a deficiency in zinc and magnesium, contribute to and cause conditions such as Autistic Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD), and dyslexia and can even decrease IQ levels (Capel et al., 1981; Grandjean and Landrigan, 2006; Al-Farsi et al., 2013; Yasuda et al., 2013). Additional chemicals like manganese, aluminium and nickel are highlighted as neurotoxic to adults (Grandjean and Landrigan, 2014). Notably, lead and cadmium present an unacceptable risk to human health and the environment. In addition, long-term exposure leads to damage to the brain and nervous system due to its neurotoxicity and

causes lifelong behavioural, developmental and learning difficulties (Centre for international Environmental Law (CIEL), 2008). Pregnant women, foetuses and children are the most vulnerable section of the population and most easily affected.

The human body can store toxic elements not only through the genes from the mother, but also through water, air, food and environmental factors (Rao, 2005). Cadmium and lead are well-known air pollutants, that are principally released as a consequence of diverse industrial processes and industries (Crosby, 1998). Despite being at low levels in the atmosphere, cadmium and lead can accumulate in the soil (Khan et al., 2017). Since these metals are constantly present in the environment, they are a growing issue in the food chain (WHO/Convention Task Force, 2007). Lead is more likely to be ingested through the consumption of contaminated water and food, which are grown in lead containing soils, and the use of leaded fuels (Peter et al., 2012). Cadmium ingestion happens mainly through the consumption of food (Järup and Akesson, 2009) or smoking. 'One cigarette contains 1-2microgram cadmium', and, moreover, 10% of cadmium is inhaled throughout the smoking period (WHO/Convention Task Force, 2007:21). Furthermore, cadmium exposure is linked to kidney damage (Lane et al., 2015) and bone damage (Järup and Alfvén, 2004), as well as causing cancer and increased mortality (Adams et al., 2012). Lead exposure, meanwhile, has neurobehavioral and developmental effects on unborn babies, children and increases adults' blood pressure (WHO/Convention Task Force, 2007). Nickel compounds are carcinogenic and poisonous (Crosby, 1998:221).

1.3 Knowledge Gap in the Field of Study

This investigation aims to establish whether a relationship exists between life expectancy and mortality rate in terms of exposure to or the possible presence of toxic metals and POPs in the hair samples of individuals living in various metropolitan boroughs of Manchester, which can adversely affect people's health. Put simply, the combined detrimental health effects of PAHs, PCBs, POPS and possible toxic metals and how these relate to life expectancy and mortality rate in a particular area is not well-established or widely-studied. This may be due to the challenge of finding a suitable sample, from or in humans, that would not be too invasive to collect and analyse and represent the pollution

levels to which the individual has been exposed. This study aims to explore the suitability of using human hair samples from individuals to create a fingerprint or profile of selected POPs and metal elements and linking that to mortality rates in the areas under investigation. As far as could be established, this kind of study is unique, particularly within the chosen region of GM.

There is a lack of epidemiologic data for the GM area linking the substance specific-hair levels with adverse health effects and mortality rate. There is little information available in the publically-available literature that is relevant to the study of environmental POPs in the hair and that explores the correlation between its level and life expectancy, nor of toxic metals in the hair which may be associated with mortality rates in different GM regions. It is important to highlight the study of Purdam (2016), *'Life on the line? Life expectancy and where we live',* which produced a map of estimated life expectancy in Manchester and so provides some information. However, Purdam's study (2016) used Office of National Statistics (ONS) rather than epidemiological data. The ONS also provide mortality rates and cause of deaths for each UK borough, which will contribute to this study by making it possible to compare and link the epidemiological data and gain evidence-based, reliable results. Therefore, this study is the first epidemiological, evidence-based study in the GM area, which may guide extrapolations and correlations regarding the existing mortality rate figures and/or indicate useful avenues for further research.

1.4 Motivation

The Greater Manchester has a long industrial history, which may contribute to the sources of organic pollutants such as PAH, PCBs and POPs, as well as toxic metals in the environment that may have a detrimental effect on human health and life expectancy. This investigation will provide some epidemiological data that may be used to inform future studies and raise the profile of the effects of POPs and metals at the local level in GM in order to contribute to public health policy. This research will use sampling methods involving human hair that will enable the detection of low concentrations of metals and POPs and allow these analytes to be measured, identified and quantified. Furthermore,

these results will be used to benchmark it against mortality rates in the different metropolitan boroughs of GM and the data from the ONS.

1.5 Aim and Objectives

The focus of this study is to obtain a fingerprint of hair clippings collected at barbers and hairdressers across GM. Such a fingerprint provide an elemental profile including potentially toxic elements, as well as a POPs profile of the hair. The data will be correlated with mortality data, obtained from governmental websites, and as such, the existence of potential links will be explored.

To accomplish this aim, the following objectives were created:

1. Determine and collect hair from nine different areas, half of which have a low mortality rate and half a higher mortality rate.
2. Compare different hair washing methods and verify the most reliable method for the rest of the hair sampling process.
3. Optimise the full digestion method for potentially toxic elemental determinations by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).
4. Optimise the analysis of POPs in hair clippings by Gas Chromatography-Mass Spectrometry (GC-MS).
5. Identify qualitatively and quantitatively the presence of potentially toxic elements and POPs in hair by ICP-OES and GC-MS, respectively.
6. Correlate the presence of selected potentially toxic elements and organic compounds with the mortality rate statistics in the collection areas.

1.6 Hypothesis: Statement

The following hypotheses were investigated:

- There is a correlation between the amount of a certain element and / or POPs and mortality rate in a specific metropolitan area.
- The differences in the concentrations within female and male hair correlate with male and female mortality rates in that area.
- The socio-economically poor areas show high metal and/or POPs concentrations.

1.7 Thesis Layout

The remainder of the thesis is structured as follows: Chapter Two focuses on a literature review of how possible toxic metals, POPs of PCBs and PAHs, adversely affect human life. Chapter Three explores the methodology for the sampling and analysis of hair. The analysed results, data and discussion relating to hair pre-treatment optimisation are presented in Chapter Four. The case study results are presented in Chapter Five in the form of graphs, figures and tables. The discussion considers the research results and their implications. Chapter Six provides a summary of findings and the achievement of the various objectives, and the thesis concludes with recommendations for future work.

2 LITERATURE REVIEW

2.1 Human Hair

Hair is important for a wide range of scientific and industrial processes, as well reflecting fashion and health within the daily life of a human being.

2.1.1 Hair as an Analysis Material

In recent years, there has been increasing interest in using hair as analytical material. The natural fibre of human hair provides useful information about the past and present conditions or exposure of the body. Hair has become a significantly important sample for human biomonitoring, as the growing need for the use of non-invasive samples has increased (Perez et al., 2012). Additionally, the structure of the hair does not undergo conformational changes. Three decades ago, scientists analysed hair samples over 2000-years-old (Wu and Wang, 1980) and over 3000-years-old (Lubec et al., 1987). Both studies indicate that the protein molecular chain has been preserved in the hair specimens for thousands of years, which has kept the hair's structure stable. Furthermore, the average hair growth and length is known to be about 1cm a month, which records one-month's information about an individual (Bader et al. 1999). Even the elements assembled in the hair are at concentrations of 10 to 50 times higher than those found in blood samples (Ambiga, 2017:21949).

Moreover, hair is described as the best biosample material, because it has a high validity when determining the elements/minerals in the body, a long shelf life and is easy to collect, transfer, and use, particularly compared to other samples like blood and urine (Jenkins, 1979; Fido and Al-Saad, 2005; Bass, 2001:472). Due to these advantages over blood and urine samples, alongside pain-free, inexpensive collection without an expert's presence, researchers are increasingly turning to hair samples for their analysis.

2.1.2 Hair related to Toxic Exposure

Over the last forty years, scientists have focused on using hair analysis to explore different areas of possible toxic exposures that cause carcinogenicity (Blaurock-Busch et al., 2014), reproductive toxicity (Dickman et al., 1998) and neurotoxicity (Bouchard, 2007), all of which are associated with pesticides or exposure to pesticides and heavy metals. Shummer et al. (2012) showed that workers' exposure to pesticides is strongly associated with their occupation. In addition, Çelik et al. (2009) linked metal exposure to people living or working near industrial areas. Hair analysis is used to screening victims for toxic metal poisoning from environmental pollutions and also to define the abnormal ingestion of trace elements in examining nutritional factors or assessing geochemical stress on people (Batzevich, 1995; Samanta et al., 2004). Hair has become a reliable biomarker tool for solving unknown matters in forensic, clinical investigation, toxicology, environmental, neurology studies (Robbins, 2012) and many more human subject areas in science.

2.1.3 Hair Construction-Chemical Level

Human hair is a complicated material, consisting of various morphological components, which are made up of diverse chemical types. It contains water, proteins, pigments, lipids and trace elements; some of these trace elements are metals and reside in the hair fibre (Robbins, 2012; Velasco et al., 2009). The main contributor to the composition of hair is protein; approximately 65–95% (Robbins, 2012:106). Proteins are the most important part of amino acid polymers. The building blocks of amino acids consist of five main elements in the hair: Carbon 51%, Oxygen 21%, Hydrogen 6%, Nitrogen 17% and Sulphur 5% (Ambiga, 2017). In terms of compounds, the amino acid composition of hair is typically Aspartic acid, Serine, Alanine, Glutamic acid, Proline, Threonine, Isoleucine, Glycine, Tyrosine, Half-Cystine, Leucine, Phenylalanine, Lysine, Tryptophan, Citrulline, Nitrogen as Ammonia, Valine, Histidine, and Arginine. Sulphur occurs in compounds such as Cysteine, Methionine, Cystine and Cysteic acid (Ward and Lundgren, 1954; Robbins and Kelly, 1970).

2.1.4 Hair Interior Structure

The interior structure of human hair is complex (Fig.2.1). Hair has two sections: the root and the shaft. The hair root, which is below the scalp, comprises a hair follicle, bulb and papilla. The hair shaft, which is above the scalp, comprises a cortex, cuticle (scales), and medulla (inner layer of the hair), as shown in Fig.2.1**.** The medulla is not always present, as will be shown in Section 2.1.5. Therefore, in this study, the hair samples are less likely to contain a medulla and, even in those that do, this is likely to be a low percentage.

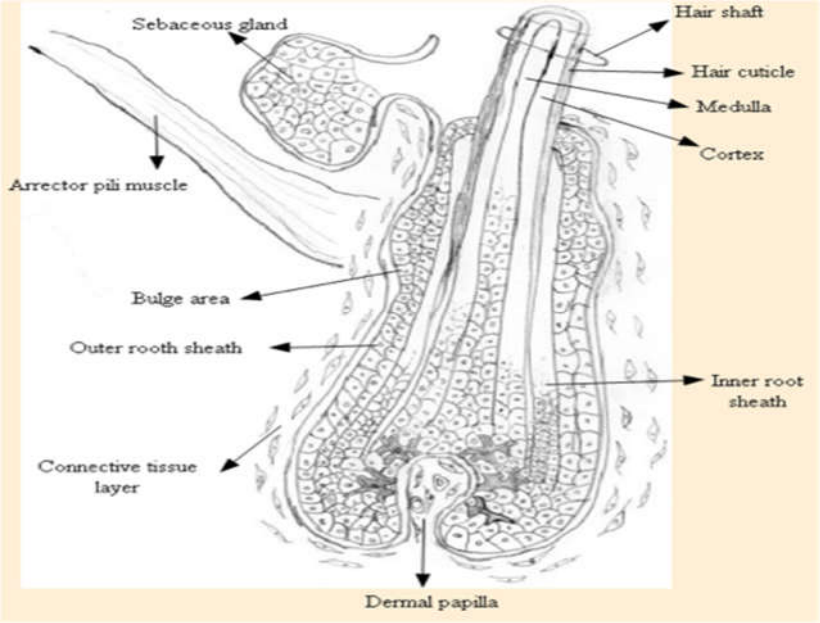

*Fig.2.1 :*Human hair and its follicle (adopted from Buffoli et al., 2014:332).

Hair fibers grow in a cycle of three steps; anagen (growth), catagen (transition) and telogen (rest) (see Fig.2.2). The growth system works independently in each follicle. For this reason, human hair does not shed at once. For example, 50-100 scalp hairs shed daily. While 90% of the scalp hair remains in the growth phase, 10% stays in transition and is in the rest phase (Wolfram, 2003: S106). Hair grows out from the hair follicles that are located on the scalp, about 15cm a year, up to a genetically determined length of around 90cm (Hoshowski, 1997:67).

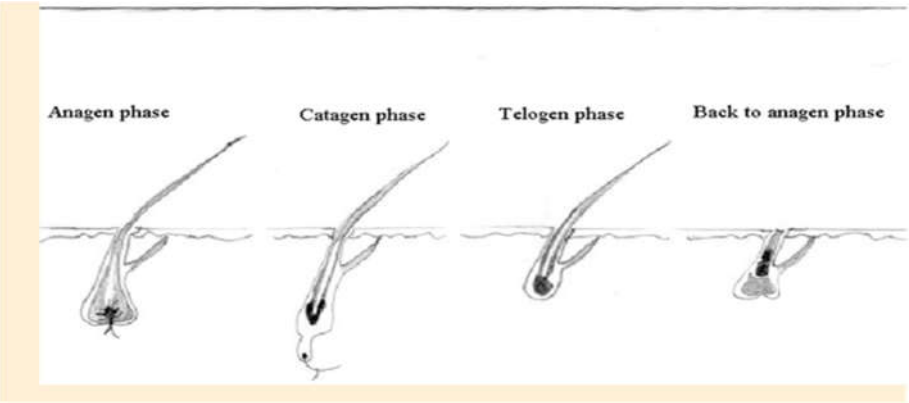

*Fig.2.2 :*Steps of hair cycle (adopted from Buffoli et al. 2014:334).

2.1.5 Ethnic Variation

The ethnic variations in hair structures have been studied widely by researchers. Human hair is categorised into three types: Asian, African and Caucasian (European). These groups are mainly determined according to the hair fibre's cross-sectional structure and diameter, shape (curly or flat), mechanical properties, chemical makeup, moisture rank and combability, as well as colour (Wolfram, 2003). Even differences in the hair surface can be seen, as exhibited in Fig.2.3–2.5. Asian and Caucasian hair fibres exhibit a circular sectional profile. African hair, on the other hand, is oval in shape and looks like a twisted rod, with focal constrictions along the hair shaft. It is noted that, based on the mechanical properties of hair fibres, African hair is weaker than Asian and Caucasian hair. Therefore, African hair fibres are more fragile under certain conditions. Asian and Caucasian hair fibres show similar behaviour during breaking, despite the differences in the diameter of the hair fibre types (Tobin, 2005:47). Schlake (2007:269) noted that:

> In order to form a flexible but almost unbreakable hair, proper keratinisation of the shaft appears to be crucial. Obviously, it is only guaranteed in the presence of sufficient and balanced keratin synthesis. For instance, deficiency of a single protein causes severe defects despite the co-expression of numerous keratins.

Overall, despite the ethnically differentiated hair types, hair keratin is alike in all human hair fibres (Tobin, 2005:47). In addition, all hair types present similar characteristics of morphology, molecular structure and chemical composition (Wolfram, 2003).

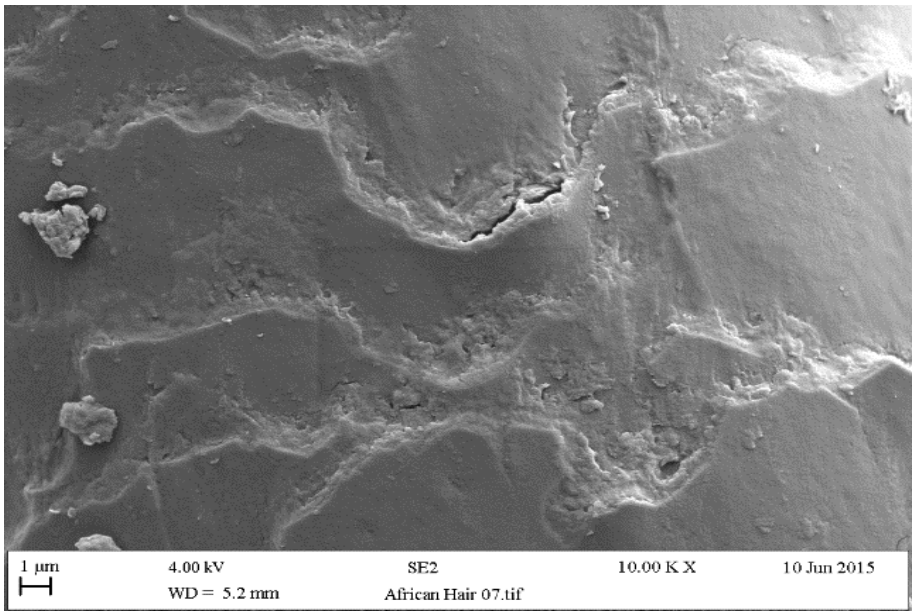

*Fig.2.3 :*The secondary electron image obtained by SEM analysis of a single African (male) hair fibre's surface. See details in the experimental Section 3.4.2.

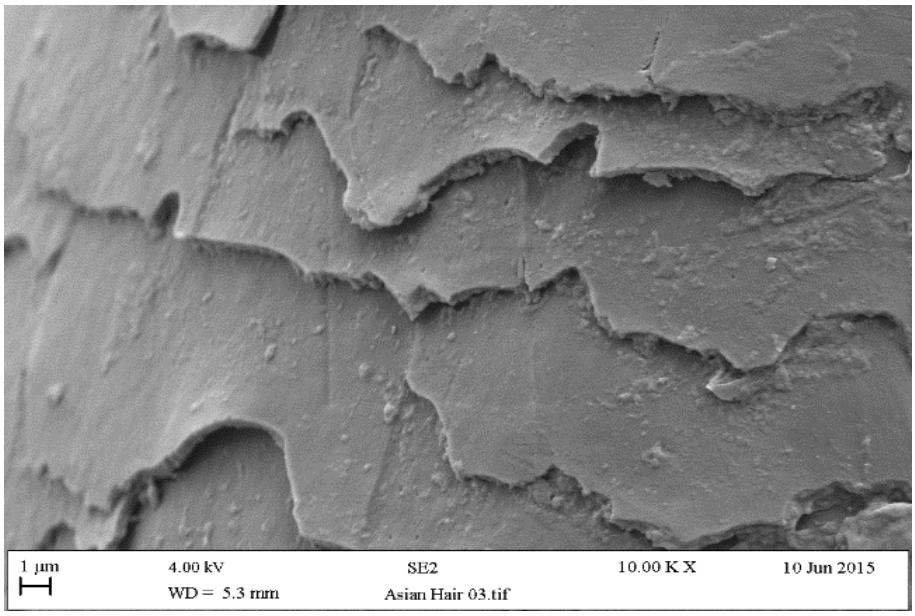

Fig.2.4 :The Secondary electron image obtained by SEM analysis of a single Asian (female-Asian-Turkish) hair fibre's surface. See details in the experimental Section 3.4.2.

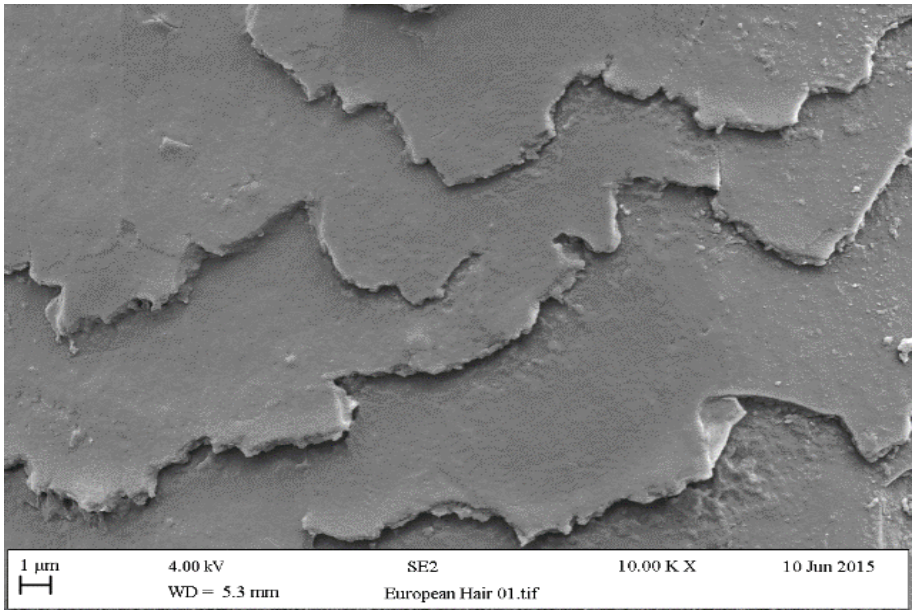

Fig.2.5 :The Secondary electron image obtained by SEM analysis of a single Caucasian (English-male) hair fibre's surface. See details in the experimental Section 3.4.2.

Fig.2.6 :A strand of (male) African hair sample exhibiting a presence of medulla in the inner layer as a dark coloured line. A strand of hair fibre analysed using a Motic BA200 microscope in transmitted light with a 400x magnifications at Manchester Metropolitan University.

Fig.2.7 :A strand of (female) Asian-Turkish hair sample indicating that there is no medulla present. A strand of hair fibre analysed using a Motic BA200 microscope in transmitted light with a 400x magnifications at Manchester Metropolitan University.

Fig.2.8 :A strand of (English-male) European/Caucasian hair sample exhibiting no medulla present. A strand of hair fibre analysed using a Motic BA200 microscope in transmitted light with a 400x magnifications at Manchester Metropolitan University.

2.1.6 The effect of Chemical and Physical Processes on Hair Properties

Chemical and physical processes decrease hair's strength and further damage the hair fiber. Chemically-treated hair reveals a larger protein shortfall than untreated hair (Hoshowski, 1997:67). Oil glands produce an oily essence called sebum, which stops hair from becoming excessively dry on the scalp. After a lengthy period, the sebum assembles, collects dirt and gives hair an oily appearance. Shampoo or soap removes the dirty sebum from the hair during washing (Tro, 2012). Repeated washing weakens the hair's strength. While the effect of shampoo increases from the centre to the outer part of the hair, the effect is limited within the cuticle. Notably, loss in the hair cortex is replaced after therapeutic or deep conditioning are applied to the hair (Hoshowski, 1997).

Human hair colour varies in nature due to two different pigments called phaeomelanin (red-orange) and melanin (dark) (Tro, 2012:376). The pigment melanin, found in the cortex of the hair, has a grainy nature (Bhushan, 2010:1). White and light blonde hair contains a very small amount of melanin. Melanin levels vary in dark blonde to brown hair, and dark hair usually has high levels of melanin. The darker the hair, the higher the levels of melanin. However, red hair has differing amounts of phaeomelanin, depending on the hair's shade (Tro, 2012). Hair pigment or texture is altered by applying chemical agents(Kuzulhara, 2013). For example, hydrogen peroxide is used to oxidise hair pigment during bleaching and permanent colouring (Hoshowski, 1997: Kojima et al., 2014). Pigments become colourless during the lightening of hair and the dye covers the surface of the hair strand when a temporary colour is applied. Conversely, permanent colouring allows dye molecules to diffuse into the hair strands (Tro, 2012:376).

2.1.7 Hair Sampling Inconstancies

The most commonly used method, as set by the International Atomic Energy Agency (IAEA) (Ryabukhin, 1976), are often given in literature, but several other techniques are also published. Collected hair samples, according to the IAEA, must be washed in the order of acetone (once)-water (trice)-acetone (once), or washed by using a Triton X solution

according to the Hair Analysis Standardisation Board (Cranton et al., 1982). These two different washing procedures, however, lead to inconsistencies between reported research/studies (Al-Farsi et al., 2013; Štupar and Dolinšek, 1996). In addition, other washing (or not treatment) approaches are also used.

Li et al. (2014) soaked hair samples in 1% detergent then rinsed them with distilled water. After this, the process was repeated twice, and the hair was then rinsed three times with deionised water. Cranton et al. (1982) washed the hair sample with acetone, and then with 1% TritonX-100 solution. Menezes-Filho et al. (2009) washed their hair sample with 1% TritonX-100 solution, but rinsed it with Millipore water (18 Ω.cm @22.8°C). Štupar and Dolinšek (1996) washed their hair sample with *n*-hexane and ethanol (ratio 1:1) and then rinsed it three times with distilled water. Schramm et al. (1992) used unwashed hair samples in their studies. Despite the guidelines, there is no standardisation regarding hair sampling/washing.

2.2 Life Expectancy

2.2.1 Studies in Britain on Life Expectancy/Death Rates

Geographically, mortality and life expectancy have been extensively studied in Britain, but the research relies on ONS data. The latest determined mortality rates and average life spans in England and Wales, according to the ONS, suggested 89 and 85 years for women and men, respectively. This is a rise in life span from 1960 to 2010 of eight years for a woman and about ten years for a man (Mills, 2012). In addition, Loopstra et al. (2016) reported that the mortality rate in England was continuing to decline between the ages of 65 to 74, which increased the number of pensioners and those over 85 years of age. Furthermore, this rise was causing cuts in financial support and social care.

In another study, Murphy and Di Cesare (2012) investigated an adult cohort mortality in England and Wales. A higher overall mortality rate was reported for males than females. Furthermore, smoking related causes of death were noted to cause high chronic obstructive pulmonary disease (COPD), lung cancer, and oral cancer for men, and cardiovascular and ischaemic heart disease (IHD) for women. Similar trends still exist in Manchester, as noted earlier in Section 1.1. Higher death rates were noted in Scotland by Mitchell et al. (2000) and lower life expectancy in northern England (Blackpool, Liverpool and Manchester) by Bennett et al. (2015) compared to the rest of England. The Greater Manchester Health and Social Care Partnership (GMHSCP) (2017) highlighted that people in GM die earlier than those in other parts of the UK. Inequalities in health were linked by the ONS data to people's daily travel, which provided an estimated journey map of life expectancy for the GM area (Purdam, 2017). Yet, to the author's acknowledge, there has not been any empirical evidence-based research in the GM focusing on life expectancy and mortality rate.

2.3 Metals

2.3.1 Theoretical Controversy when Defining Metals

There is a variation in the use of the term of 'toxic metals' in many research studies. A considerable amount of literature has been published on 'heavy metals', which refers to 'toxic metals' and have a negative impact on living organisms. However, there is an inconsistency in defining heavy metals due to the use of different densities. Moreover, no link exists between physiochemical concepts and density, nor the toxicity or ecotoxicity accredited to 'heavy metals' (Duffus, 2002:804). The term 'heavy metals' is, however, used loosely and lacks scientific validity. Hence, academics/scientists offer diverse definitions of 'heavy metals' (Bjerrum, 1936; Smith and Nordberg, 2015). For example, according to Bjerrum (1936) metals that have a greater than 3g/cm^3 elemental density are classified as heavy metals. In contrast, Järup (2003) stated that heavy metals are mostly defined as having elemental density levels higher than 5g/cm^3. Banfalvi (2011:11) described it as:

> Heavy metals are...trace elements with ≥ 3g/cm^3 densities, have some biological functions at low concentrations and cause toxic effects at higher than physiological concentrations.

Hence, the term '"heavy metals" [is] -a meaningless [expression]' because Duffus (2002) states that, according to the chemical definition of 'metal', most elements are likely to be categorised as metals. In this investigation, the use of the term 'heavy metals' refers to metals that exceed the concentrations of physiological levels and which create health issues and toxic effects.

2.3.2 Legitimative Requirements Post Definition

Studies worldwide have highlighted the adverse effects of metals on living organisms, particularly human beings (Järup, 2003; Krantz and Dorevitch, 2004; Wirth and Mijal, 2010). Heavy metal monitoring is inconsistent on the international and often national level, and even the public health laws are not always appropriately enforced (Mamtani et al., 2011).

2.3.3 Metals and Their Impact on Life Expectancy and Mortality Rate

Recently, the production of metals and metal alternatives has increased dramatically worldwide. Thus, the exposure of the population living in such areas close to environmental pollutants raises potential health concerns. In addition, the higher mortality ratios in Huelva province as compared to the rest of Spain has contributed to an increased concern among the population about the potential adverse health effects posed by environmental pollution (Benach et al., 2004). Huelva province in Spain is well-known for its past and current mining activities, and a clear link was found between environmental pollution as a result of metal contamination and the local population's health. Table 2.1 shows a comparison of the average metal values found in this study based in Manchester with those published in the literature. It is clear that, while there are some similarities, the concentrations of several of the minor and trace metals found in the hair samples in

Manchester exceed those of the other studies described in the literature, in some cases quite significantly (Ni, Pb, Cu, Fe and Zn, for example).

2.3.4 Hair as a Biological Marker

Table 2.1 provides an overview of metal levels in hair found in this study and compared to two articles found in literature. It is observed that there are good agreements with what our study has found and what have been reported in these two articles, with the exception of Cr, Mn and Pb. All three these elements are of toxic and / or carcinogenic concern.

Table 2.1 :Hair Metal Concentration in this study compared to those in literature

		This study (mg kg^{-1})		**Biolab Medical Unit, 2012**	**Davis and Miller, 2010**
		Female	**Male**	**Composite (µg/g)**	**Male (µg/g)**
Trace elements	**As**	0.38	0.73	<1	0.3
	Cd	0.03	0.03	<0.10	0.01
	Cr	*0.38*	*0.40*	0.10-1.50	0.16
	Mn	*0.73*	*1.18*	0.20-2.00	0.25
	Ni	0.90	0.68	<1.40	0.52
	Pb	*3.96*	*7.70*	<2	1.90
Minor elements	**Al**	22.4	31.0	50	20
	Cu	21.1	19.4	10-100	13
	Fe	28.5	50.7	5-30	13
	K	97.7	127	50-300	85
	Mg	*216*	80.7	60-160	28
	Na	200	158	50-1000	347
	P	133	169	100-200	159
	Zn	258	279	160-240	138
Major elements	**Ca**	1800	700	200-2800	609
	S	37700	41400		

2.4 POPs

2.4.1 Legal Requirements

Due to POPs posing risks to health and the environment, under the Stockholm Convention established in 2001, the signatory countries were legally obliged to begin to remove POPs from production, use and emissions (United Nations Environment Programme, 2001a,b). This applied mostly to 12 frequent toxic POPs, the so-called 'dirty dozen' (Table 2.2), being targeted and legal measures then put into force in 2004 (Kaiser and Enserink, 2000).

Table 2.2 :Sources of POPs

Pesticides	Industrial chemicals	Unintentional by-products
Aldrin*	Hexachlorobenzene*	Hexachlorobenzene***
Chlordane*	Polychlorinated biphenyls (PCBs)*	Polychlorinated dibenzo-p-dioxins/furans ***
DDT**	Hexabromobiphenyl *	PCBs***
Dieldrin*	Hexabromocyclododecane *	
Endrin*	Hexa & hepta bromodiphenyl ether *	
Hexachlorobenzene*	Hexachlorobutadiene*	Hexachlorobutadiene***
Mirex*	Pentachlorobenzene*	Pentachlorobenzene***
Toxaphene*	Perfluorooctane sulfonic acid (PFOS), & its fluoride salt (PFOSF)**	
α-hexachlorocyclohexane	Polychlorinated naphthalenes*	Polychlorinated naphthalenes***
β hexachlorocyclohexane	Tetrabromodiphenyl ether*	
Chlordecone*	Decabromodiphenyl ether*	
Heptachlor*	Chlorinated paraffins (SCCPs)*	
Lindane*		
Pentachlorobenzene*		
Pentachlorophenol*		
Technical endosulfan*		

12 initial POP's (dirty dozen) and the most recently added chemicals. *Eliminate (Annex A), ** Restrict (Annex B), ***Reduce (Annex C) (Adapted from United Nations Environment Programme, 2001a and 2001b; and Stockholm Convention Secretariat, 2017).

Most recently, in 2017, an additional 16 POPs were added to those for ultimate removal and decrease (highlighted in the Table 2.2). The legislation on the POPs Regulations in England and Wales came into force on the December 3rd 2007.

2.4.2 POPs and their Impact on Life Expectancy and Mortality Rate

A correlation between air pollution and yearly mortality rate was found in some cities of the United States, and smoking and air pollution were particularly connected with mortality rates due to lung cancer and cardiopulmonary diseases (Dockery et al., 1993:1755). The hospitalisation rates were reportedly higher for coronary heart disease, particularly for myocardial infarction (35.8% and 39.1%, respectively) (Sergeev and Carpenter, 2005: 756) in relation to residence near areas contaminated with POPs and other pollutants, such as areas close to the Hudson River compared to uncontaminated areas. Therefore, contaminated locations clearly pose a risk of exposure and onset of diseases associated with mortality rate. In addition to the general adverse effects of POPs, 17 PAHs were outlined in the studies as carcinogens (Rengarajan et al., 2015), which are also analysed and investigated in the current study. These PAHs were: Benz(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Chrysene, Dibenz(ah)anthracene, Indeno (1,2,3) and Pyrene.

Furthermore, PCBs are also known carcinogens. They can alter immune system functions and cause adverse alterations to the nervous system, skin, thyroid, and sex steroid hormonal systems. Other bodily areas which can be affected are the liver, kidneys, pancreas, and the cardiovascular system. As a result of these actions on multiple organ systems, humans who are exposed to PCBs are at an increased risk of cancer, infections and reduced cognitive functions accompanied by adverse behavioural effects, hypothyroidism, infertility, ischemic heart disease, hypertension, diabetes, liver disease, as well as giving birth to infants of lower than normal birth weight. Some of these adverse effects, such as IQ deficits that result from perinatal exposure (Jacobson and Jacobson, 1996), are irreversible and cannot be treated, while many other diseases, such as cancer, can be treated successfully if diagnosed early (Carpenter, 2006).

2.5 Analytical Techniques for assessing Metal and POP Exposure

POPs and excessive heavy metal consumption/exposure through the environment and unknown sources have become the subject of several studies where hair was analysed using various techniques. Hence, the most frequently-used techniques reported in the studies were: X-Ray Fluorescence (Khudzari et al., 2013), Scanning Electron Microscopy with Energy Dispersive X-Ray (SEM-EDS) (Fresnais et al., 2015), Atomic Absorption Spectroscopy (AAS) (Ilyas and Shah, 2017), Graphite Furnace AAS (GFAAS)(Nancy and Miller-Ihli, 1988; Baysal and Akman, 2011), High Performance Liquid Chromatography with Fluorescence detection (Toriba et al., 2003), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), (Chojnacka et al., 2010), ICP-Mass Spectroscopy (ICP-MS), (Chojnacka et al., 2010; Manduca et al., 2014), Nano scale Secondary Ion Mass Spectroscopy (Nano SIMS) (Hallégot et al, 2008), Gas Chromatography-Mass Spectroscopy-Mass Spectroscopy (GC-MS/MS) (Wielgomas et al., 2012; Grova et al, 2018), Headspace Solid Phase Microextraction (HSSPME) and GC-MS (Tzatzarakis et al., 2014), and Gas Chromatography – Mass Spectrometry (GC-MS) (Appenzeller et al., 2017). In this study, GC-MS was chosen for the analysis of extracted hair samples for POPs, while ICP-OES was employed for the analysis of metal elements following microwave digestion of the hair samples. Fewer previous studies were reported on POPs (Covaci and Schepens, 2001; Appenzeller et al., 2012; Schummer et al., 2012; Król et al., 2013), while several were published on metals (Smart et al., 2009; Herman et al., 2013; Mehra and Thakur, 2016; Baloch et al., 2017; Bell et al., 2018) in human hair. It is therefore a proven approach and, in this study, has been selected to determine if the results of the hair analysis could be correlated to death rates in GM.

2.6 Contribution of the present study

While previous studies reported the successful analysis of different metals and POPs in human hair, the focus of the current work will be on attempting to link such results to death rates in various metropolitan areas in GM. The emphasis will thus be shifted from method development and analysis to seeking to identify a correlation with human health in the

chosen areas of the study. Therefore, this study may open up a new route for researchers and authorities to explore, particularly at a local level, the implications of pollution and the related health effects which impact on the longevity and mortality rate of the general population living in particular areas.

3 METHODOLOGY

A report by Greater Manchester Health and Social Care Partnership (GMHSCP, 2017) stated that health inequalities in the population shapes Greater Manchester (GM)'s future and that people in GM die earlier than those living in other parts of the UK. To investigate this statement, the levels of potentially toxic metal elements and persistent organic pollutants (POPs) in hair were measured using a number of techniques to see if the values correlate with the death rates. It has been demonstrated in the literature review that the procedures need to be standardised to achieve accurate and reliable results. This includes ensuring the following: correct sample collection, optimising consistent hair sampling protocols, hair treatment methods, extraction and digesting techniques, quality control and quality assurance procedures. For that purpose, this study explored optimising the methods of hair sample treatment and analysis and applied this to a case study by testing specific metal elements and POP analytes that are related to human health and their connection with mortality rate in the GM region.

3.1 Chemicals and Laboratory Equipment

For the sample preparation, Triton X-100, acetone and methanol were used to clean the hair samples. For the elemental analysis, analytical grade reagents and trace select mineral acids were used (Fluka, 69% HNO_3). A multi-elemental standard was used for the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) calibration standards (Inorganic Ventures, Christiansburg, USA). The multi-elemental standard solution consisted of: 1000mg/L : Al, Ca, Fe, K, Na; 500mg/L: Cu, Mg, P, S; 200mg/L: As, Cd, Co, Cr^{3+}, Mn, Mo, Ni, Pb, Zn (matrix; 5% HNO_3 in 250ml).

For the POPs analysis with GC-MS the following reagents were used, all of HPLC grade (99%, ACS reagent): Acetonitrile (ACN), Sodium Acetate(powder, bioreagent, SIGMA), Magnesium Sulphate (anhydrous, SIGMA), Florosil® (60-100 mesh, for column chromatography), Hexane (ACROS Organics, UK), Benzo(g,h,i)perylene (97% chemical

purity (D12,98%) 20 µg/ml in Toluene-D8, from Cambridge Isotope Laboratories (Andover, USA).

Deionised water (Millipore water; 18.2 $M\Omega cm^{-1}$ at 22.8°C) was provided by a Synergy® UV water purification system (Merck, UK) and used throughout the investigation.

To reduce contamination, all of the equipment, such as the glassware and plastic vials, beakers, stoppers, measuring cylinders and glass jars were soaked in a 10% Nitric acid (HNO_3) bath for 24 hours prior to analysis for elemental (metal) profiles. After exposure by washing with HNO_3, they were rinsed six times with deionised water (DIW) and allowed to air dry before usage.

VWR Ergonomic high-performance Eppendorf pipettes were used and calibrated by weighing selected water volumes (Supplementary data available Appendices-A and B) where the correlation between the mass and volume provided R^2 values close to 1). The analytical balance was calibrated prior to use by applying the 'OHAUS, slo-A-weigh' procedure (R^2 value 1, supplementary data available in Appendices-C).

3.2 Sampling Methodology

Over the past four decades, hair has become an important sample material for researchers. According to Jenkins (1979:3),

> If hair samples are collected properly, cleaned and prepared for analysis correctly, and analysed by the best analytical method using experienced personnel, the data are reliable.

Head hair in particular is regarded as the best body region for samples to analyse and a sample size of between 50–100 participants is accepted as satisfactory (Tilstone et al., 2006:181). However, Süße et al. (2010) reported that there was no statistically significant difference between other body part hair samples and scalp hair samples. Hair sampling is a non-invasive method. Because hair samples are collected after the hair has been cut and

are not pulled from the scalp, the collected hair samples are not subjected to the Human Tissue Act of 2004. Another important main point is that, the moment that the hair is cut, the biohazard diminishes very quickly as hair lice feed on human blood and, in the absence of the blood, adult lice will die within a day or two, while hatchlings die within hours (Scot and Tomas, 2000; Zinsser, 2007). Therefore, by the time of the sample preparation, potential infestation by head lice is not a factor.

3.2.1 Sample Collection

Method Optimisation

The human scalp hair fibres were collected from barbers in Stockport and hairdressers in Salford and Manchester in May 2014. In this study, the samples are classified into Sample A and Sample B (which are female and male hair samples, respectively). Sample A was collected from hairdressers (mainly styling women's hair) and Sample B from barbers (mainly cutting and styling men's hair). It is estimated that the sample consisted of 120 type-A and 150 type-B clippings. The hair clippings were swept up from the floor and collected after a week, put into sealable plastic sample bags and labelled with date, location and sample type. The reported age of Sample A was 3-75 years, and that of Sample B 2-90 years.

It is common practice for early-age children to go with their parents to have their haircut. Mothers may take their sons to have their hair cut at a hairdresser and fathers may take their daughters to barbers to have their hair cut. However, the barber and hairdresser shop owners reported that their samples did not include different sex hair. Furthermore, the barbershop proprietors highlighted that the hair samples contained beard and moustache hair, but at a minimum level. As is known from the literature, hair samples are categorised as Caucasian (European), Asian or African. However, hairdressers or barbers did not report the presence of African type hair during the sample collection process.

The samples in this study were not collected as strands of individual hair, but were rather a mixture of many individuals' hair, which were pooled as hair cuttings over a week. Therefore, the samples are classified as a bulk sample. These bulk samples were subjected to elemental analysis to optimise the best hair treatment method as described in Section 3.3. Part of the optimization process was continued 2 years later on some old composite samples to determine if unwashed and deionized water washing would yield the best result. These samples differed in homogenisation and composition from the first batch and should only be compared in terms of repeatability of the data obtained after the different washing methods have been applied.

Case Study

The case study was carried out after optimising the best method for pre-hair treatment before analysis. The collected (bulk) hair samples were donated by different barbers and hairdressers in GM between May and June 2017, including the boroughs of Bolton, Bury, Manchester, Oldham, Rochdale, Salford, Stockport, Trafford and Wigan (Fig.3.1).

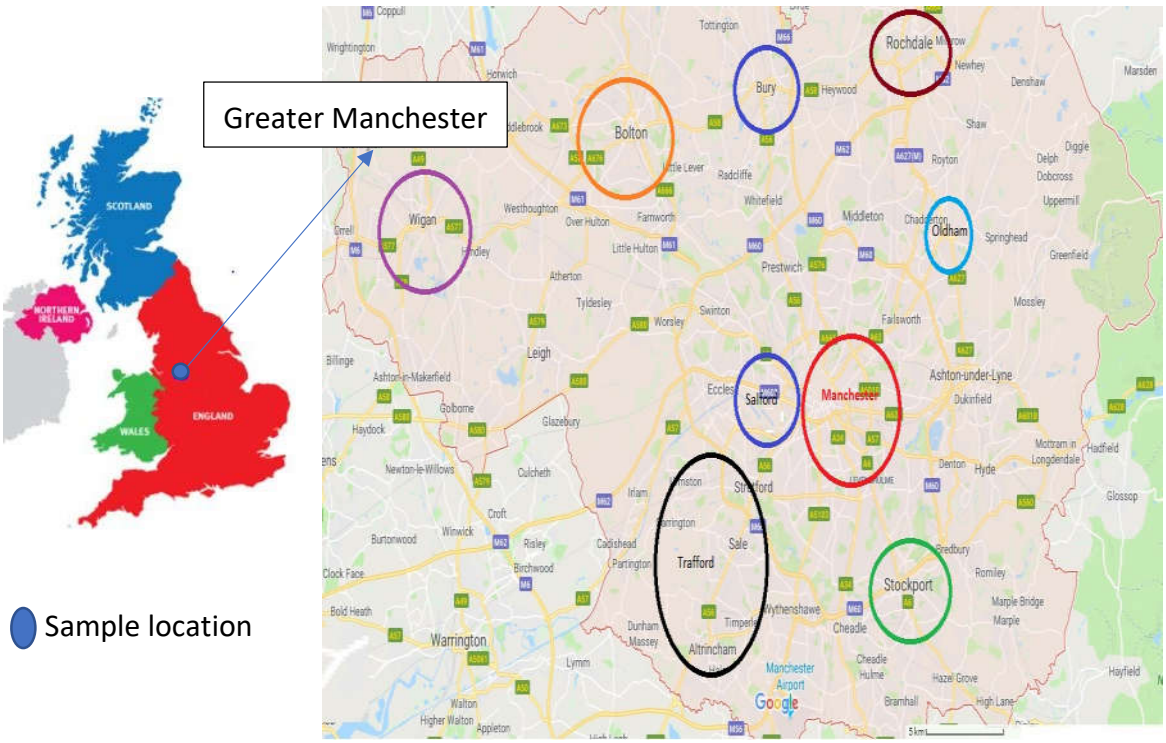

Fig.3.1 :Map showing the location of the hair collection sites for the case study.

The barber and hairdresser shops were mainly selected based on the willingness of the proprietors to participate in the study. In general, the shops were in the central part of the town, where two composite samples were collected per area. Further information on the sample locations and the number of shops involved can be found in Appendices-D. Sample requests were made during visits to shops and a letter was handed to the proprietors (see Appendices-E) requesting permission particularly to collect pooled hair cuttings. The hair samples collected from barbers and hairdressers, were classified into female (Sample A) and male (Sample B) specimens. The general assumption was that the composite samples would represent the population of the specific area. According to the shop owners the number of clients per sample varied from 50 to 130 (see the distribution of the hair shops in Fig.3.2), which equate to ~1,114 type A samples and 1,551 type B samples. The reported youngest age was 3 (female) and 1 (male), and the oldest was 85 in both groups. However, the shop owners highlighted that their clients were mainly adults. Visual images and further information can be found in Appendices-D.

Fig.3.2 :Distribution of the hair shops from which the Sample A and B hair clippings were collected in Greater Manchester.

The samples were collected in a similar way to that described in Section 3.2.1, apart from the fact that they were stored in paper bags to ensure that the POP analysis is not compromised. After reaching the laboratory, the samples that needed to be analysed for metals were left in the paper bags but double bagged using sealed plastic bags and re-labelled. The samples that needed to be analysed for POPs were transferred quickly into the methanol rinsed glass jars and re-labelled at the Manchester Metropolitan University (MMU) laboratory on the day of collection.

3.2.2 Sample Cleaning

The following section describes the sample preparation. All of the hair samples were kept in an air-controlled environment at a MMU laboratory at 21°C ± 2°C.

Washing Procedure Optimisation

The previously collected and labelled samples, A and B, were taken to the laboratory, weighed and transferred into eight plastic sealed re-labelled bags. The total weight of Sample A was 833 grams and that of Sample B 947grams. Each sample bag underwent visual, physical, and foreign body examination during the three-step sample cleaning process (see Fig.3.3).

Step 1:

The visual and physical observation: The hair samples were placed on a bench lined with clean white paper in the laboratory. All plastic sieves and tweezers used were cleaned by a Nitric acid wash to reduce contamination.

Sample A was very clean, and comprised black, burgundy, red and dark brown hair. It was reported by the hairdressers that ~60% of the hair was chemically coloured. No foreign objects were found.

Sample B was mainly natural black and grey hair, with some hairs that were bright brown. Furthermore, there were also white, blond and hazel coloured hair samples; whether these

were natural or chemically-coloured could not be confirmed without additional information.

There was evidence that Sample B contained some foreign objects: a black plastic string, food debris, dead head lice, small particles like sand, small stones, some wool fibres, wood chippings, a broken human nail and leaves.

Step 2:

Clearance of noticeable foreign bodies and dust: Sample A was clean, but Sample B contained solid dandruff flakes, sticky-tape, broken paint chips in addition to what was found during the first step. The dust was removed using a plastic sieve and magnifying glass. During this step, some of the head lice, sand and dust passed through the sieve allowing larger foreign objects to remain.

Step 3:

Removing objects invisible to the naked eye by magnifying glass: This was achieved by the aid of plastic tweezers. At this stage, wood chippings, small pieces of black plastic (possibly part a comb) and leaves were identified. These were all removed from Sample B.

After the cleaning, Samples A and B were cut into 2-5mm pieces over a period of 15 days. It took three days to cut 303 grams of the female hair. The hair samples were cut using acetone wiped stainless scissors to reduce possible contamination regarding the metal element profile. The hair cutting process was time consuming and required planned labarotory scheduling to manage it. The bulk samples (types A and B) were then ready for the homogenisation procedure. The same procedure was followed for the second optimisation (unwashed / deionised water wash).

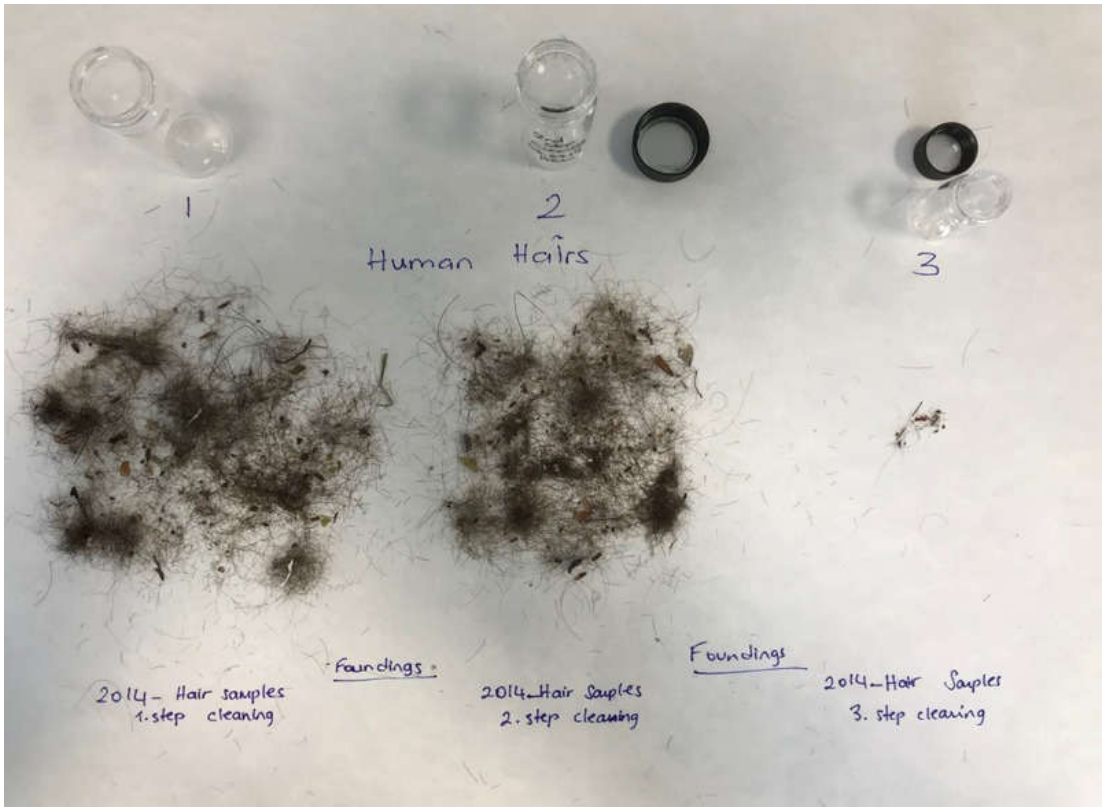

Fig.3.3 :The three-step cleaning process to reduce foreign objects.

Case Study

The preparation of the case study hair samples followed the same cleaning procedures for the metal analysis as explained in Section 3.2.2. The cleaning steps for the POP analysis were slightly different. During the cleaning of the hair samples, a methanol rinsed stainless steel sieve and metal tweezer were used to cut the hair to < 5mm. The hair samples were then washed with DIW and air dried, yielding 39 samples of A and B, ready for the homogenisation procedure.

3.2.3 Sample Homogenisation

The cut bulk samples were reduced to an appropriately sized testing sample using the coning and quartering technique. This technique is accepted broadly as a sample dividing method (Prichard and Barwick, 2007). Furthermore, the applied technique allowed representative sub samples to be obtained.

Sample Preparation Optimisation

The entire hair sample was placed on a clean white paper on a bench, thoroughly mixed, then piled into a cone. The cone was flattened, and the hair sample was divided into eight portions (Fig.3.4). The opposite quarters were mixed. This process was repeated ten times. Finally, the samples were divided into eight piles and the two opposite quarters of this sample were taken as a subsample for the pre-hair treatment method. The rest of the sample was re-labelled and kept for further analysis, if needed.

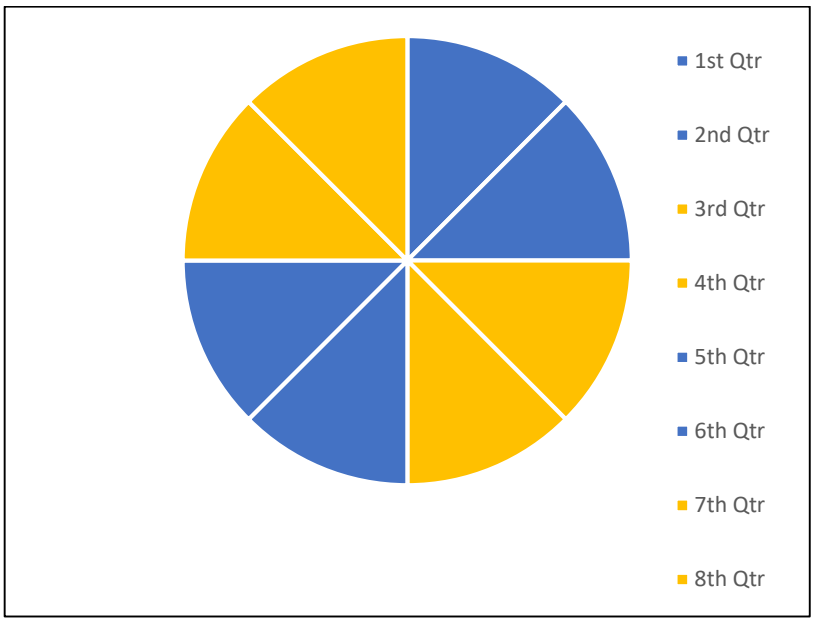

Fig.3.4 :Coning and Quartering method. Each portion represents hair samples.

150 grams of Sample A and B, respectively, were cut further to a size of 0.5mm. To optimise the pre-hair treatment method, a composite hair sample (Sample C) was prepared. Sample C comprised a subsample of homogenised Samples A and B (50 grams each). In addition, to ensure that the samples were as homogenous as possible, a Graffin Flask Shaker (volts 200/250, watts 60, model Griffin and George Ltd, Great Britain) was used to agitate the sample for 20 minutes.

Case Study Analysis Considerations

The coning and quartering method was used, as explained above. Subsamples were taken to determine the POPs and metals. The rest of the samples were kept for further analysis, if required. 50 grams of Sample A and Sample B, respectively, were cut to smaller than 0.5mm in length and mixed to allow a representative subsampling (the sample sizes were verified by scanning electron microscopy images, Fig.3.8 and Fig.3.9). Agitation was performed by a Graffin Flask Shaker for 20 minutes on the sub samples. All samples were weighed, labelled and transferred into the methanol rinsed glass jars for POPs analysis and into 10% Nitric acid washed glass jars for the elemental analysis. The samples were then ready for the next steps, where a Modified QuEChERS technique or Microwave-Assisted Digestion method was employed.

3.3 Hair Pre-Treatment Optimisation

This section describes the optimisation of the best washing method, where various approaches in other studies were applied to the samples collected. According to the literature, there are several treatment methods available prior to analysis: washed or unwashed. The most widely-used washed methods were:

1. Triton X-100: According to Wright et al. (2006), hair samples were sonicated in ~10ml of 1%Triton X-100 solution in a 50ml beaker for 15 minutes, then rinsed with DIW and oven-dried for 24 hours at 70°C. The use of a 0.01% Triton solution is also recommended by the Hair Analysis Standardisation Board (Cranton et al., 1982).

2. Water-Acetone-Water (WAW): This method was claimed and used as an International Atomic Energy Agency (IAEA) standard in some studies, such as Baloch et al. (2017). However, IAEA standards are slightly different than described by Ryabukhin (1976). The method involved washing the hair samples three times in the order of ultrapure water-acetone-ultrapure water, and then oven-drying them at 60°C.

3. Acetone-Water-Acetone (AWA): This method was described by Ryabukhin (1976:13) as an International Atomic Energy Agency (IAEA) hair sample washing standard. High purity acetone and water were recommended. Hair samples were washed once with acetone, then three times with water and again once with acetone. After the washing procedure, all of the samples were air-dried at room temperature in a dust-free, clean location.

4. Unwashed: The earlier study of Chittleborough (1980:53) supported a 'no-wash policy for hair analysis', on the ground that this approach is holistic and realistic for obtaining valid information about the concerned presence of exogenous and endogenous trace metals.

Statistical analysis was used to confirm the best treatment type (Appendices-F:1).

3.3.1 Wash Methods

To compare the various wash methods with each other, the following methodology was adopted:

1. Triton X-100 wash, 2. Water-acetone-water wash, 3. Acetone-water-acetone wash, 4. Unwashed, and 5. a water wash (DIW only).

1: 0.2 grams of each of the samples (A, B and C) were soaked in acid-washed 100ml beakers with ~10ml of 1%Triton X-100 solution, put in an Ultrasonic Bath (at 230 volts, 200 watts; XUBA3 Grant model, UK) at 40℃ for 30 minutes. The detergent was discarded using a pipette and rinsed six times with DIW. This process was replicated twice, and then the samples were oven-dried (Carbolite model) at 70℃ for 35 minutes. Wright et al. (2006)'s method as adopted was thus slightly altered here.

2: 0.2 grams of each of the samples were washed with water (DIW)-acetone-water (DIW) (WAW) as reported by Baloch et al. (2017). However, while following the same washing order, the samples were rather air-dried in a dust-free area.

3: 0.2 grams of each of the samples were washed three times in acetone-water (DIW)-acetone, (AWA), adopting the IAEA standard (Ryabukhin, 1976). The only difference was a that three washes with acetone were applied also.

4: 0.2 grams of unwashed hair samples were not subjected to any washing process. In a second phase step 4 and step 5 (described below) were performed on a composite hair sample, as well as female and male hair, to determine if washing the sample with DIW would be the best approach as the results previously (2014) showed significant variation with the use of Triton and the acetone washes. Due to the time lapse between the first set of data and the second phase it should be stated that the samples used in phase one and phase two were different and not from the same batch.

5: 0.2 grams of each hair sample was washed with DIW until the discarded water was clear. In this study, three wash were sufficient.

3.4 Sample Preparation for Instrumental Analysis

This section describes the sample preparation for the elemental and POPs' profiles.

3.4.1 Microwave-assisted Acid Digestion (MAD) and QuEChERS Method

MAD

Several studies reported using an acid digestion method for the hair sample preparation (Wright et al., 2006; Menezes-Filho et al., 2009; Blaurock-Bush et al., 2011; Shin et al. 2015; Baloch et al., 2017), and mostly microwave-assisted acid digestion (MAD). MAD is a cost effective closed-vessel digestion method, which applies high pressure to the samples to be digested. It is accepted as a robust sample prep technique for Atomic Absorption (AA), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyses (Smita et al., 2013; Eça et al., 2014; Uddin et al., 2016).

MAD was used to dissolve all of the acid soluble metals of interest; i.e, Aluminum (Al), Arsenic (As), Cadmium (Cd), Calcium (Ca), Copper (Cu), Chromium (Cr), Iron (Fe), Lead (Pb), Magnesium (Mg), Manganese (Mn), Nickel (Ni), Phosphorus (P), Potassium (K), Sodium (Na), Sulphur (S) and Zinc (Zn) for ICP-OES analysis. To reduce possible contamination, 55 ml size PTFE (polytetrafluoroethylene) vessels were first cleaned in a laboratory

dishwasher, soaked in a 10% HNO_3 (70% standard laboratory grade) acid bath for 24 hours, and then rinsed with DIW. The final stage entailed using a microwave oven (MarsExpress-5 version 194AO2, CEM Microwave Technology, UK), employing the same digestion profile as summarised in Table 3.1. Following the cleaning cycle, the PTFE tubes were rinsed with DIW and air-dried for the sample preparation.

0.2 grams of each sample were weighed accurately on a Sartorius analytical balance and then transferred into acid washed PTFE tubes with the addition of 5ml HNO_3 (69%). The samples were then introduced into a Cem MarsExpress 5 instrument (see Table 3.1). Blanks were prepared and treated in the same way.

*Table 3.1 :*Microwave-assisted Acid Digestion Method for the Hair Sample.

The MAD profile was adapted from CEM Microwave Technologies UK (personal communication with Chris Mason).						
MAD HAIR SAMPLE PROFILE						
Stage	Power	Digestion	Ramp	℃	Hold	Cool down
	Max	%		Control		
1	1200W	90	10 min.	90℃	5 min	
2	1200 W	100	10 min.	170℃	10 min	
3						30 minutes

Following the digestion, the samples were pressure relieved in the fume cupboard prior to transferal into a volumetric flask and then diluted with DIW and made up to a volume of 25 ml. Peter et al. (2012:771) made a significant suggestion to prevent nebuliser clogging; i.e., that cloudy solutions needed filtering using Whatman paper 1 and 40. However, 55mm diameter filter paper (Fisher Scientific) was found to be optimal. The cloudy solutions were further filtrated (PTFE 25 mm, 0.45µm NSTR) prior to transferring the samples to vials for ICP-OES measurements.

QuEChERS Extraction Method

After the study of Anastassiades et al. (2003), QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) has become a popular analysis method worldwide, including applied extraction and clean-up methods. The application of QuEChERS is still in the development stage for a diversity of analytes, but the method is successful for the analysis of mycotoxins and Polycyclic Aromatic Hydrocarbons (PAH) in complex matrices (González-Curbelo et al., 2015). The preparation of hair samples was reported in most of the recent studies as using a QuEChERS method for pesticide analysis by Gas Chromatography-Mass Spectroscopy (GC-MS) and Ultra-high-Performance Liquid Chromatography-Mass Spectroscopy/Mass Spectroscopy (Lehmann et al., 2018a and 2018b). A QuEChERS extraction method (Haimovici et al., 2016; Megson et al., 2016) was modified to prepare hair samples for the Gas Chromatograph-Mass Spectrometer (GC-MS) to determine the POPs (Fig.3.5), which were investigated in this study. The sampling was explained in Section 3.2. and a modified QuEChERS extraction technique is shown in Fig.3.5. The QuEChERS technique combines two extraction procedures: first, the hair samples were extracted and then cleaned by dispersive Solid Phase Extraction (dSPE). For the extraction and clean up, acetonitrile and Hexane treatment was used and the extracted solvents were blow down by nitrogen evaporators (Fig.3.6).

The following POPs analytes of 17 PAHs: Naphthalene, 2-Methylnaphthalene, Acenaphthylene, Acenaphthene, Anthracene, Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(g,h,i)perylene, Chrysene, Dibenz(a,h)anthracene, Fluoranthene, Fluorene, Indeno(1,2,3-cd)pyrene, Phenanthrene, Pyrene, and 7 PCBs: PCB28, PCB52, PCB118, PCB138, PCB153, PCB180, PCB209 were measured. D10 Anthracene +D12 Benzo(g,h,i)perylene was used as a recovery standard and D10 Phenanthrene as an injection standard.

According to the National Statistical Release Index of Multiple Deprivation (IMD) (Gill, 2015: 15), Manchester is ranked the top most deprived local authority district in England, followed by Rochdale, Salford and Oldham, ranked at 21, 22 and 29 respectively.

Furthermore, GM is ranked fifth and features among the top 10% of most deprived areas nationally. In two of the underlying domain indices reported (health deprivation and disability), it is ranked at 31.3% and, for the living environment, at 7.2% (Gill, 2015:19). Of the total of 2.8 million people living in GM, 680, 000 GM inhabitants are included in the 10% most deprived areas (GMHSCP, 2017).

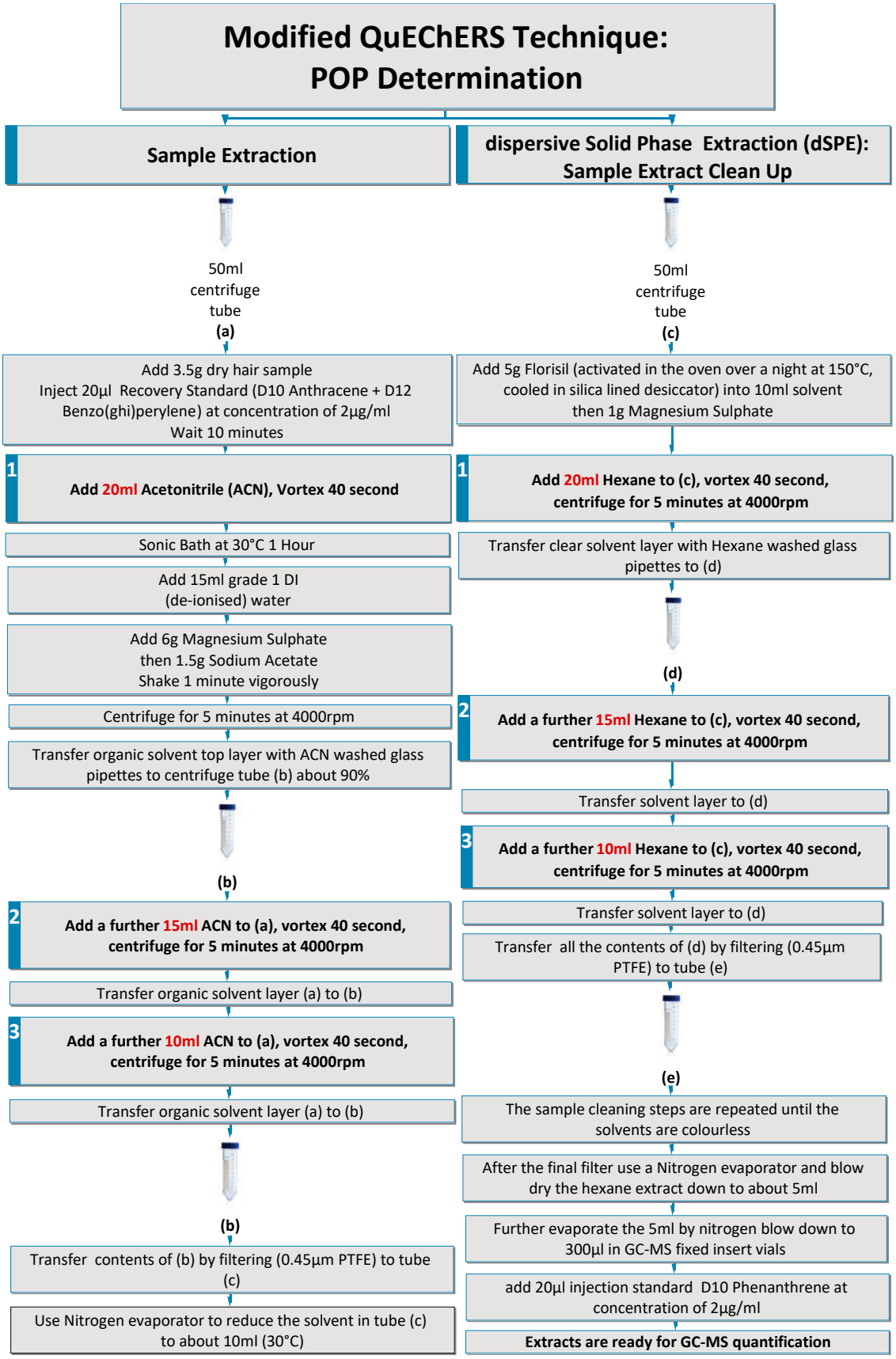

Fig.3.5 :A modified QuEChERS technique for POPs determination in hair samples.

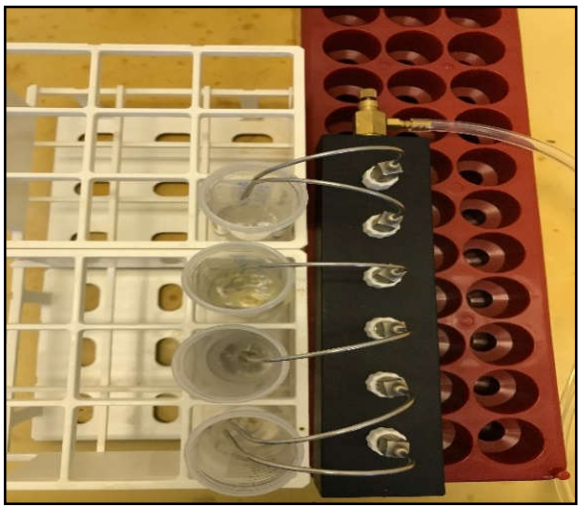

Fig.3.6 :Extracted solvents blown down by nitrogen evaporators.

3.4.2 Instrumental Analysis

Scanning Electron Microscopy (SEM)

SEM forms high quality images of surfaces, which are invisible to the naked eye, by using a thinly focused electron beam, which can yield magnifications of up to 100,000X, while allowing reasonable flexibility regarding sample preparation (Hui, 2006).

SEM (JEOL Ltd manufactured, JSM-5600LV model, with SEM Control User Interface version 5.60) was used in this investigation, firstly for analysing single hair treads to illustrate different ethnicity hair samples, as well as to verify the sample size (from sample-types A and B) for representative sampling.

1. SEM (see Section 2, Fig.2.3-2.5) operated at an acceleration voltage of 4.00 kV, for a gold platinum coated sample (sputter coated, see Fig.3.7) was used to examine Asian, African and European hair samples have the scale size of 1µm and resolution images at 10 000X magnification. The Secondary Electron Detector (Everhart Thornley) is used to image samples at a working distance of 5.2mm for African hair and 5.3mm for Asian and European hair, as shown in Section 2.1.5. These three different types of hair samples were examined at MMU under the same settings.

*Fig.3.7 :*The sputter coated samples of three different (Asian, African and European) hair strand samples.

2. SEM analysed sputter-coated samples type-A and B (Fig.3.8 and 9) at an acceleration voltage of 4.00 kV, with the scale size of 300 and 100µm, and resolution images at 65 and 140 X magnification (female and male respectively). The Secondary Electron Detector (Everhart Thornley) was used to image the samples at a working distance of 9.9mm. The images below show that the longest length cut hair size was 4.019mm (Sample type-A), with an average length of 1.018mm (Sample type-B). Therefore, the average size of the hair cuttings prepared is ≥1mm.

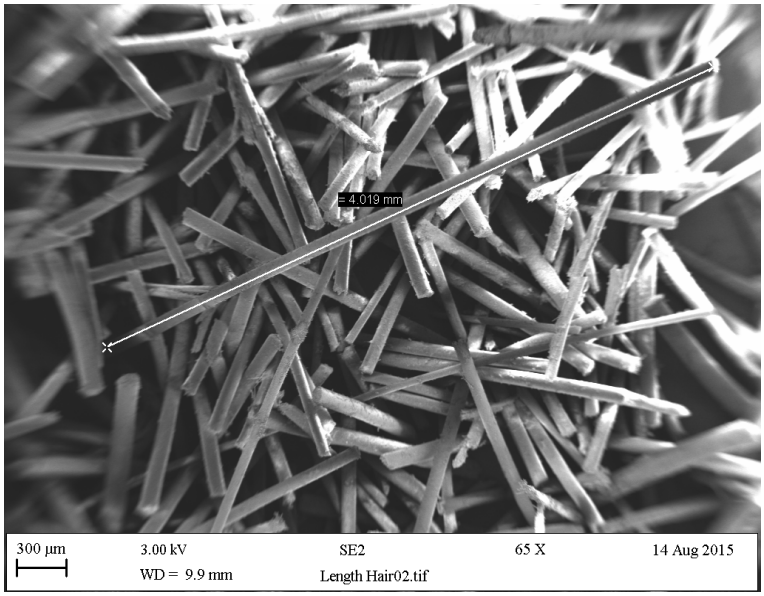

Fig.3.8 :The Scanning Electron Microscopy image of Sample Type-A female hair size.

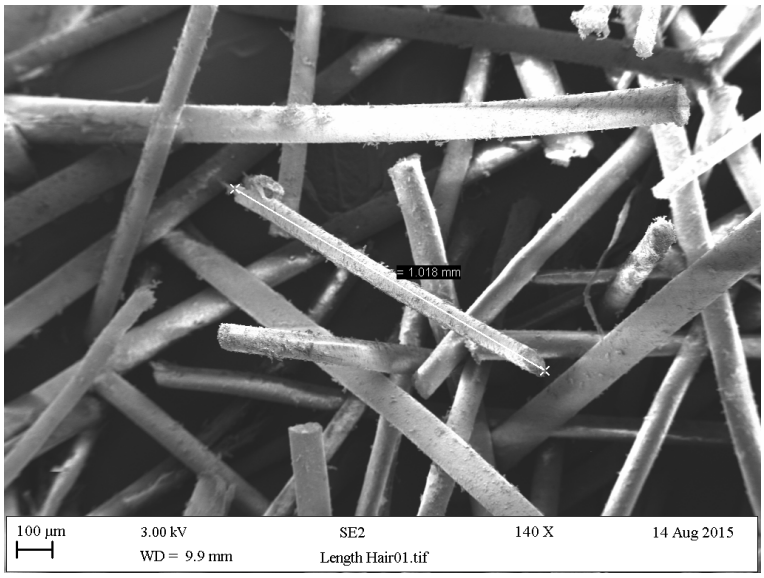

Fig.3.9 :The Scanning Electron Microscopy image of Sample Type-B male hair size.

Microscopy

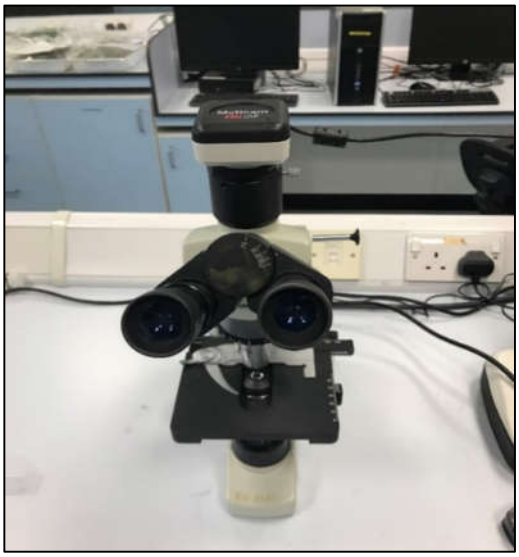

Fig.3.10 :The Motic BA200 model microscope used in the investigation.

African, Asian and European hair samples were examined for their structure under the microscope at MMU. A Motic BA200 model microscope with Moticam 10 Mega Pixel camera and software of Motic Images Plus 2.0ML (Motic Live Imaging Module) was used to explore the inner layer of the different types of hair fibres in section 2.1.5. (see Figures 2.6-2.8).

Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)

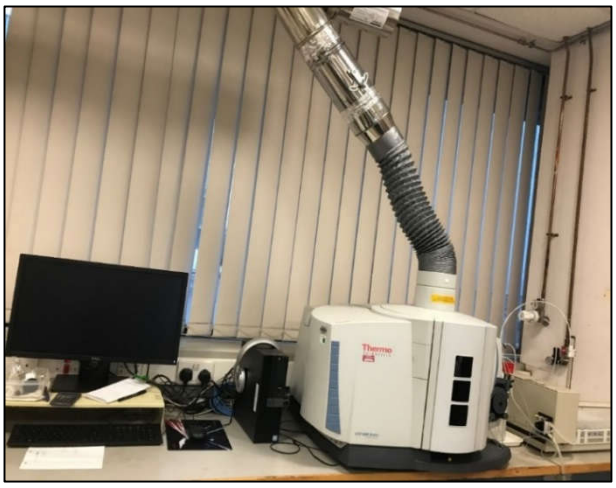

Fig.3.11 :The ICP-OES spectrometer utilised in the investigation.

ICP-OES is a useful analytical technique for determining trace metals. It allows multi-elemental analysis and can measure 70 elements simultaneously (Harris, 2010). However, it typically requires two separate sample preparation steps. A Thermo-Fisher ICP-OES was used in this study to investigate the metal profile in hair. The Thermo Scientific iCAP 6300 DUO model ICP-OES settings are summarized in Table 3.2. The instrument settings for Na, Mg, Ca and P elements were done in a radial view, while the rest of the elements were measured in an axial view configuration. The ICP-OES operating parameters, including the elements and their analytical lines (wavelengths), are tabulated in Table 3.2.

Table 3.2 :Instrumental Operating Conditions for ICP-OES

Parameter	**Value**
RF Power	1250W
Rump Rate	50rpm
Auxiliary Gas Flow	0.5 L/min
Nebuliser Gas Flow	0.55 L/min
Elements and Analytical Lines (at wavelength, nm)	(1670) Al; (1890) As; (4226) Ca; (2144) Cd; (2677) Cr; (3247) Cu; (2404) Fe; (7698) K; (2802) Mg; (2576) Mn; (5895) Na; (2316) Ni; (1174) P; (2203) Pb; (1820) S; (2062) Zn

Calibration standards and blanks were prepared in 20% HNO_3. The calibration standards ranged from 0.01, 0.05, 0.1, 0.5, 1ppm. The same calibration range was used for the method optimisation and the metal profile in the case study. Each sample was analysed in triplicate and each had five instrumental repeats. All readings were then averaged to provide the values employed in the data analysis. For the blanks, the same analytic procedures were followed as for the MAD hair samples.

Gas Chromatography-Mass Spectroscopy (GC-MS)

Fig.3.12 :The GC-MS spectrometer used in the investigation

The GC-MS analysis was performed on an Agilent 7890B Gas Chromatogram (GC) directly coupled to the Single Quadrupole MS system of Agilent with a 7693 Autosampler and a 5977B Mass Selective Detector (MSD). The operation parameters of the GC-MS are provided in Table 3.3. In addition to the 39 extracted samples and ten sample blanks from the QuEChERS method, a Hexane blank and six calibration standards were also prepared. The calibration standards ranged from 1, 5, 20, 100, 400, 750 pg/µL for PAHs and, for the PCBs, these were 1, 5, 20, 100, 400, 1000 pg/µL. During the analysis, Hexane and Calibration Standard 3 (CS3) were run after each ten samples to check for accuracy. During the analysis, each sample analysis was run in triplicate and then averaged to provide a value in the data evaluation.

The qualitative analysis program of Agilent, i.e. MassHunter Workstation Software, B05.00, was used for the GC-MS data. The software supports the data and screens the compounds by targeted MS (Agilent Technologies, 2012). In this study, the compounds and their retention times were known in the MS, and those obtained from the analysed hair samples were compared against the standard values to identify the compounds present. In particular, the PAHs and PCBs peaks were extracted from the chromatographs after the

CS3's retention time had been determined. The area of each pollutant's peak for the samples and calibration standards were calculated by manual integration.

Table 3.3 :Operating Parameters for the GC-MS analyses used in the investigation

Operating Parameters	**Operating Value**
Column	Agilent J&W capillary DB-5MS, 30m x 0.25mm x 0.25µm film thickness
GC Run time	15 min
Oven Temperature/ Program	Start at 60°C, 3 min (hold time), Ramp at 20 °Cmin^{-1} to 300°C
Carrier Gas	He (Helium) 1m$Lmin^{-1}$
Injection	Split, 1 µL
Injection Temperature	280°C
Split Ratio	20:1
Split Flow	20m$Lmin^{-1}$
MS Detector Transfer Line Temperature	300°C

Table 3.4 :Mass and retention times of the POPs compounds.

PAHs			**PCBs**		
Compounds	**Mass Compound**	**Retention Time (Rt)**	**Compounds**	**Mass Compound**	**Rt**
Naphthalene	129.1	3.7	PCB28	258.0	10.1
2-Methylnaphthalene	143.1	4.4	PCB52	289.0	11.0
Acenaphthylene	153.1	5.5	PCB118	323.9	13.4
Acenaphthene	155.1	5.8	PCB138	361.8	15.8
Fluorene	167.1	6.7	PCB153	361.8	16.6
Phenanthrene	179.1	8.9	PCB180	395.8	18.5
Anthracene	179.1	9.1	PCB209	494.0	22.9
Fluoranthene	203.1	12.6			
Pyrene	203.1	13.3	**Recovery Standards**		
Benz(a)anthracene	229.1	17.8	**Compounds**	**Mass Compound**	**Rt**
Chrysene	229.1	17.9	D10 Anthracene	189.1	9.0
Benzo(b)fluoranthene	253.1	21.6	D12 Benzo (g,h,i)perylene	289.2	26.5
Benzo(k)fluoranthene	253.1	21.8			
Benzo(a)pyrene	253.1	22.7	**Injection Standard (IS)**		
Benzo(g,h,i)perylene	277.1	25.9	**Compounds**	**Mass Compound**	**Rt**
Indeno(1,2,3,-cd)pyrene	277.1	26.6	D10 Phenanthrene	189.1	8.9
Dibenz(a,h)anthracene	279.1	26.1			

3.5 Limits of Detection (LOD) and Limits of Quantification (LOQ)

ICP-OES

The LOD and LOQ (Tables 3.5 and 3.6) were calculated from the ten filtered and unfiltered method blanks (ppb) for the ICP-OES. The formulas used were:

LOD = (3*standard deviation(s))/m for the signal of the blanks,

LOQ = (10*standard deviation(s))/m for the signal of the blanks (Harris, 2010).

Table 3.5 :ICP-OES LOD and LOQ at ppm (μgL^{-1}). Filtered Blanks

Elements	**Al**	**As**	**Ca**	**Cd**	**Cr**	**Cu**	**Fe**	**K**
LOD	7.8	8.5	129	0.4	2.8	4.9	9.9	234
LOQ	26	28	429	1.4	9.3	16	33	779
Elements	**Mg**	**Mn**	**Na**	**Ni**	**P**	**Pb**	**S**	**Zn**
LOD	6.8	0.3	194	3.6	6.4	11	11	4.0
LOQ	23	0.9	646	12	22	36	36	14

Table 3.6 :ICP-OES LOD and LOQ at ppm (μgL^{-1}). Unfiltered Blanks

Elements	**Al**	**As**	**Ca**	**Cd**	**Cr**	**Cu**	**Fe**	**K**
LOD	7.6	6.1	73	0.3	1.7	2.3	4.9	200
LOQ	25	20	242	1.0	5.5	7.6	17	668
Elements	**Mg**	**Mn**	**Na**	**Ni**	**P**	**Pb**	**S**	**Zn**
LOD	21	0.3	246	2.5	3.3	7.0	15	3.1
LOQ	68	1.0	820	8.2	11	23	51	10

GC-MS

The LOD (Table 3.7 and 3.8) for the instrument is estimated based on calibration data. Calibration Standard 1 (CS1) was the lowest concentration which could be consistently detected (Armbruster et al., 1994). The formula used here is y=mx, where y=peak area, m= slope, x= concentration (Harris, 2010).

Armbruster et al. (1994:1234) reported that 'LOQ is defined as the concentration…[where the] quantitative value is within ±20% of the target concentration'.

Table 3.7 :GC-MS LOD of various PAHs given in ppb (pg/uL).

PAHs	**LOD (pg/uL)**	**PAHs**	**LOD (pg/uL)**
Acenaphthene	2	Phenanthrene	6.1
Acenaphthylene	3.4	Pyrene	2.1
Fluorene	1.4	Benzo(a)pyrene	1.9
2-Methylnaphthalene	5.3	Benzo(b)fluoranthene	3.6
Naphthalene	1.5	Benzo(k)fluoranthene	<LOD
Anthracene	<LOD	Chrysene	<LOD
Benz(a)anthracene	11.1	Benzo(g,h,i)perylene	7
Fluoranthene	2.3	Dibenz(a,h)anthracene	4.9
Indeno(1,2,3-cd)pyren	3.1		

<LOD means below the Limit of Detection

Table 3.8 :GC-MS LOD of various PCBs given in ppb (pg/uL)

PCBs	PCB28	PCB52	PCB118	PCB138	PCB153	PCB180	PCB209
LOD	<LOD	3.8	4.8	6.2	4.6	5.0	6.9

3.6 Recovery Controls: GC-MS

A common practice for checking the precision of the technique and the efficiency of the extraction during the analysis of organic compounds in samples is to calculate the amount of compound recovery. For the purpose of this analysis of PCBs and PAHs in hair samples, the following controls were run:

Table 3.9 :Recovery results of sample analysed by GC-MS

Recovery%	**CS1**	**CS2**	**CS3**	**CS4**	**CS5**	**CS6**
Benzo[ghi]perylene	96.5	100.2	93.6	93.1	108.7	107.8
D10anthracene	92.7	99.6	88.3	91.4	113.2	114.8

As the results summarised in Table 3.9 show, good and complete recoveries were obtained from the samples spiked with the two calibration standards (CS). However, during the hair analysis, the samples spiked with standards yielded very poor or no recovery at all. For example, the female hair samples from Bolton gave a recovery of one standard of only 9%. The recovery of Benzo[ghi]perylene in the blanks was 1- 6%, whilst an average 29.7% of the spiked D10anthracene was recovered from the analysed hair samples. However, Sadeghi et al. (2016) determined 13 PAHs in mineral water using a similar method to the one employed in this study and, using D10anthrecene as an injection standard, noted recoveries of 71-90%, which is much higher yields than the present study.

3.7 Statistical Analysis

To confirm the best hair treatment optimisation, the hair treatment types vs elements were statistically analysed to explore any significant differences. A one-way ANOVA was conducted between Hair Treatment Types vs Elements and post hoc comparison using the Tukey test. A one-way ANOVA (Kerr et al., 2002) was also conducted between the hair wash options vs elements vs sample types (female, male and composite) and for Cr and Mn, post hoc comparison using the Tamhane test. For Zn, a post hoc comparison using the Tukey

test was applied. The well-known T-test was applied to compare washed and unwashed hair samples. A one-way ANOVA was conducted between the areas vs elements and significant differences at the $p<0,05$ level for nine conditions and a post hoc comparison using the Tamhane test was completed. Furthermore, an independent sample t-test for gender vs elements as well as shops vs elements was applied. Statistical differences were reported only when there was a significant difference found in all data sets. The SPSS 22 program was used for all statistical analysis. For the statistical results, see Appendices-F:1 and 2.

4 Results and Discussion of the Hair Pre-Treatment Optimisation

4.1 Hair Pre-Treatment Optimisation

As mentioned in the literature review, the optimal hair washing method is not standardised, in the prior research, although several methods have been developed. One objective of this thesis was to compare different hair washing methods and verify the most reliable method for the rest of hair sampling process. As part of the investigation, the hair samples were analysed after various washing treatments, as well as in an unwashed state, to determine whether the metal analyses differed substantially as a result of the pre-treatment.

The washing treatments were applied to female, male and composite (mixed female and male) hair samples, as explained in Methodology, Section 3.3. The analysis procedure is provided in the Section 3.4.1–3.4.2. The mass concentration of various metals, i.e. Cadmium (Cd), Chromium (Cr), Manganese (Mn), Nickel (Ni), Lead (Pb), and Zinc (Zn), was determined for the hair pre-treatment optimisation. The results obtained regarding these elements' values are presented in Appendices-G. The concentration levels were calculated and given in ppb, or $\mu g kg^{-1}$, for all elements. The results obtained are presented in the following figures. The detailed statistical results are added in Appendices F to confirm the analytical results.

The steps taken for hair pre-treatment optimisation in the first phase were as follows:

4.1.1 Unwashed, Triton Wash, Water-Acetone-Water (WAW) Wash and Acetone-Water-Acetone (AWA) Wash

The analysed Male, Female and Composite hair samples were previously treated by washing in low concentration Triton solution (Triton), as well as water-acetone-water (WAW) and acetone-water-acetone (AWA) combinations. Changes in the hair pre-treatment methods for these four conditions were compared. In each case, the five

elements in the Male, Female and Composite hair samples were measured and compared. The results obtained for these four possibilities of washing treatment methods are shown in the following Figs.4.1– 4.4. (Key: UM = unwashed male, TM = Triton wash male; WAWM = water-acetone-water male; AWAM = acetone-water-acetone male; UFM = unwashed female, TFM = Triton wash female; WAWFM = water-acetone-water female; AWAFM = acetone-water-acetone female; UC = unwashed composite, TC = Triton wash composite; WAWC = water-acetone-water composite; AWAC = acetone-water-acetone composite).

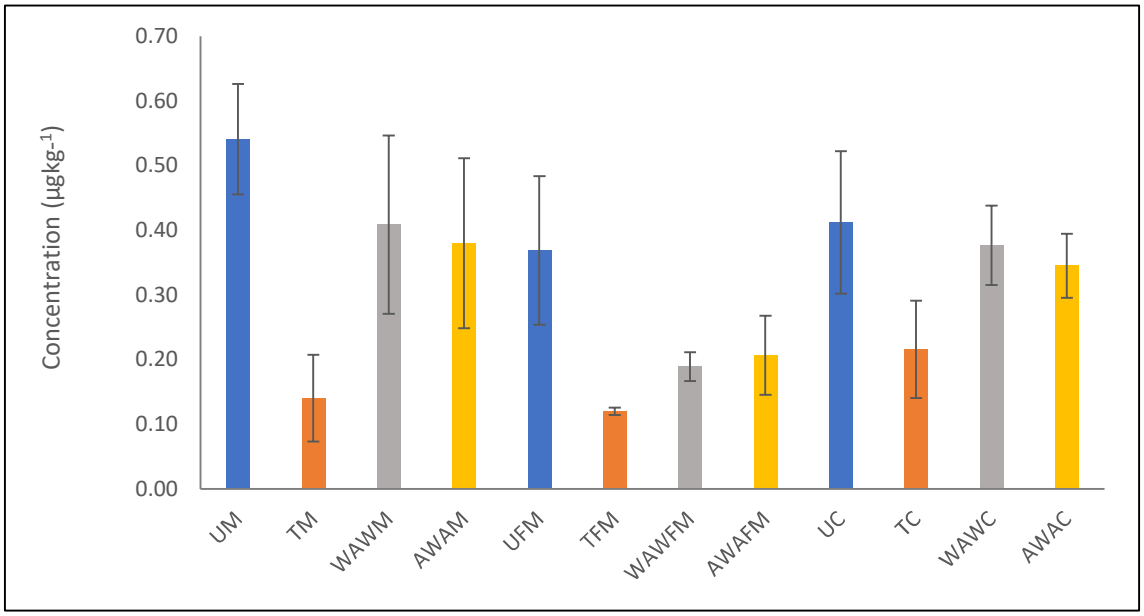

Fig.4.1 :The effect of various washing treatments on the Cr analysis in the different hair sample types.

In Fig.4.1 illustrating the Cr values, a difference can be seen between the WAW and AWA washes of the female compared to the male hair and composite hair samples that were treated in the same manner. In the other two pre-treatments no statistical differences between the hair types were observed for the Cr analysis. In general, the male hair sample yielded larger Cr analysis values than the female hair. The Cr concentration was high in all hair treatment types for males, with the exception of the Triton treatment. It is clear from the data that the unwashed pre-treatment yielded overall the highest concentration.

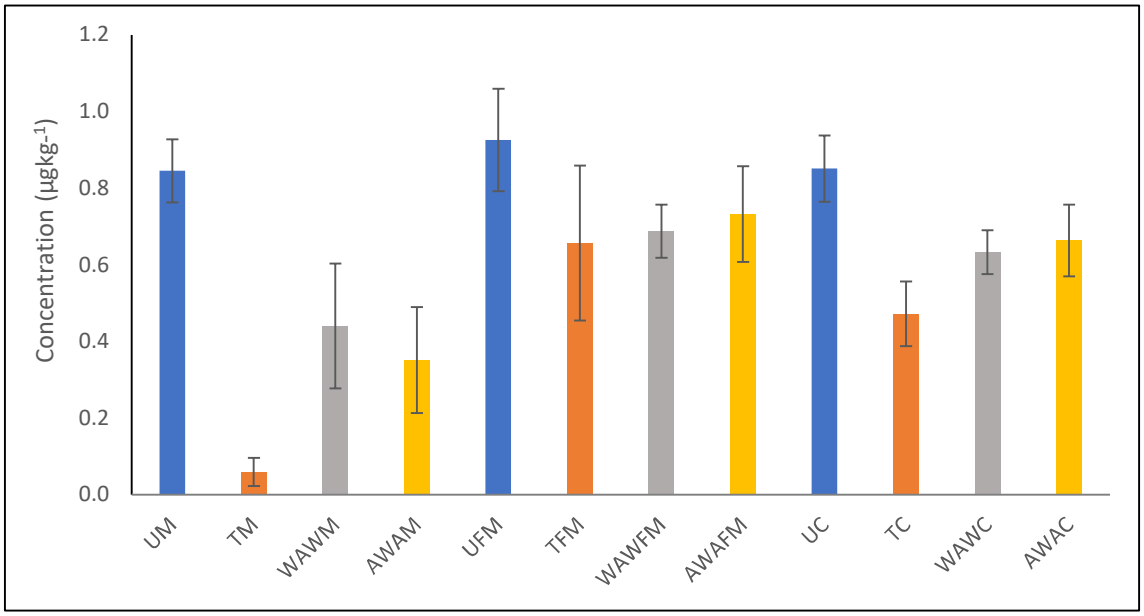

Fig.4.2 :The effect of various washing treatments on the Ni analysis in the different hair sample types.

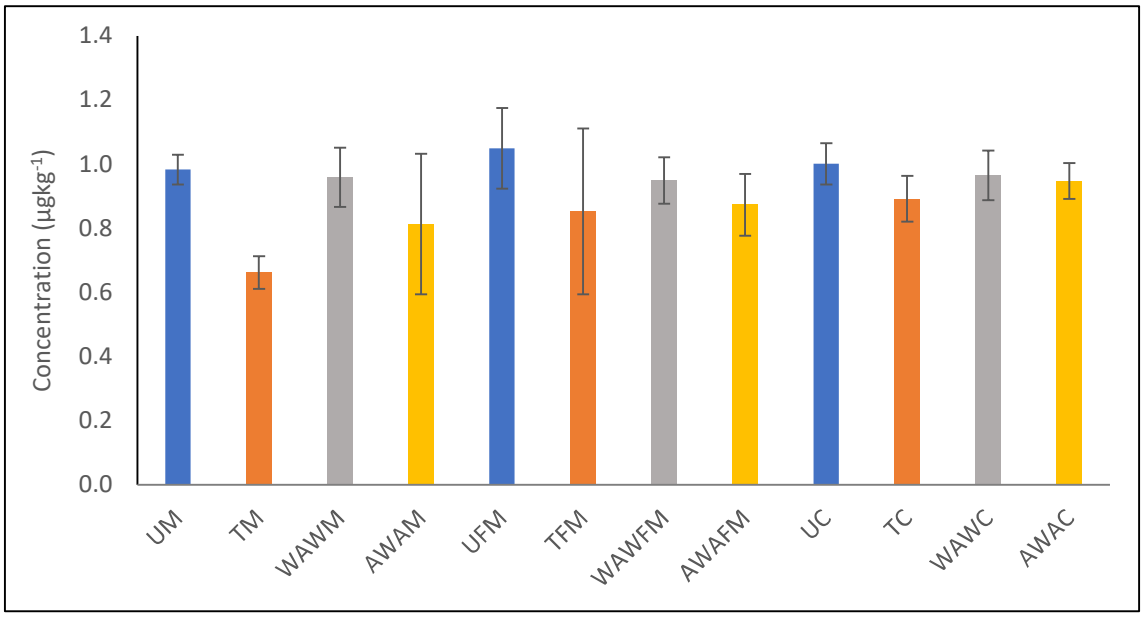

Fig.4.3 :The effect of various washing treatments on the Pb analysis in the different hair sample types.

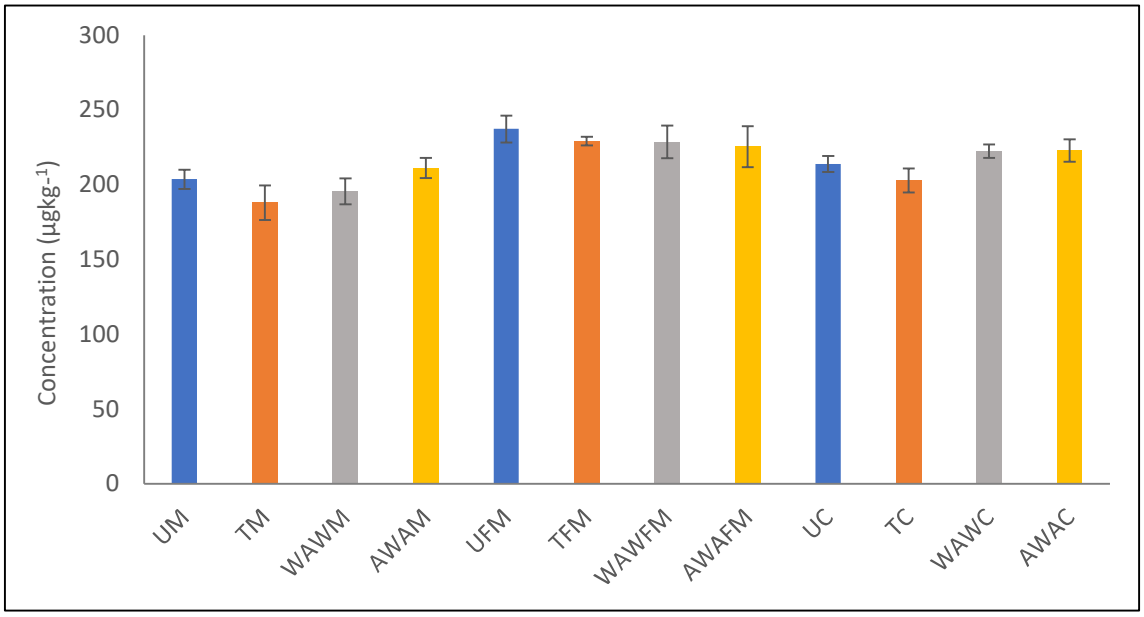

Fig.4.4 :The effect of various washing treatments on the Zn analysis in the different hair sample types.

The graphs in Fig.4.1–4.4 illustrate the effect of all of these washes and no washing at all on the specific elemental content of various hair samples from the male barbershops, female hairdresser shops and composite samples of both male and female hair in the same area. The data is presented with error bars, which are representative of the standard deviation on ten subsamples with five instrument repeats. The Cd data is not presented as the values were mostly below the limit of detection. In addition, the Mn levels were also in general below or very close to the LOD and clear evidence of an unhomogenised sample was displayed, and was therefore also eliminated.

In Fig.4.2 the results for the Ni contents in the female and composite hair types are, within experimental error, the same for the different pre-treatment washing methods performed. The male hair washed with the different treatments did not produce similar results, with the Triton was performing the worst and producing very low results in comparison with the other methods. It once again seems as though no treatment produced consistently the highest result and that the other treatments do not produce reproducible results.

Fig.4.3 shows the results of the Pb analyses after various pre-treatments/washings for the three different hair sample types. A similar pattern than what was observed with the Ni results presented in Fig.4.2 are displayed with the exception that also the male hair data are within experimental error the same. There are no clearly distinguishable differences between the type of hair sample analysed or the different washing treatments, but there is a marginable higher Pb concentration with no treatment.

Fig.4.4 present the Zn values for the different hair types after the various washing treatments. The WAW wash yielded a lower Zn content in the male hair sample than in the composite and female hair types samples. Otherwise, the Zn content in all three hair sample types with all the washing and treatment methods are every time within experimental error the same. No clearly distinguishable differences exist between the different hair samples and they are more or less similar in their Zn content.

Assarian and Oberleas (1977) reported that the Zn result for unwashed samples was higher than for a Triton wash. Sen and Das Chaudhuri (2001) reported that non-ionic-acetone (Triton X-100 is a non-ionic detergent) is the best wash method for hair analysis when determining lead. Despite the Pb content being more or less constant in Fig.4.3 for all hair treatment methods, the Triton wash for male hair samples was different from the rest of the treatments as well as compared to the values obtained for the composite and female hair samples. Overall, the results, particularly those in Fig.4.2, highlights that a Triton wash often yielded different analysis results from the rest of the treatment types. The triton wash treatment showed distinct characteristics in general in all the figures for each element of all sample types as well as the comparison with other treatment type results, particularly in the case of the Ni and Pb analysis. The reason why the Triton wash results are so different and far lower or absent/below the detection limit compared to the rest of the cases is unclear and requires further investigation. Therefore, due to inconsistent results for the Triton washes, this treatment was not considered as a successful pre-treatment method for hair before analysis.

The results obtained and described above highlight the fact that the chosen hair treatment method could affect the results and that the same element can vary based on the chosen wash method (Bass et al., 2001). For example, Štupar and Dolinšek's study (1996) reported different Pb results due to the effect of either washing or not washing hair, despite the fact that the same bundle of hair was used. Interestingly, as shown in Fig.4.3, the Pb values obtained display a more or less constant trend.

A comparison of Figs.4.1–4.4 indicates that Zn is, by far, the metal present in the highest concentration in the male and female hair samples. Its concentration is two orders of magnitude larger than those of the next two highest-level metals in the hair; namely, Ni and Pb.

Despite the similar tendency seen in Cr and Zn metal analyses between WAW and AWA washing treatment types in Fig.4.1 and Fig.4.4, the WAW and AWA washing treatments yielded different Mn (not shown), Ni, and Pb values in Fig.4.2 and 4.3, respectively. Based on the concentration distribution in the unwashed sample types for the Ni, Pb and Zn (Fig.4.2–4.4, respectively), the unwashed method was chosen as the preferred pre-hair treatment method. This initial phase was followed by a second phase to compare a De-ionised Water (DIW) wash with the Unwashed method, which was shown to be preferable in the first phase. This second phase took place a couple of years later.

4.1.2 Unwashed and De-ionised Water (DIW) washed comparison of Hair Samples.

To distinguish between the Unwashed and Washed hair treatment methods, the male, female and composite hair samples analysed for various metals (Fig.4.5–4.10).

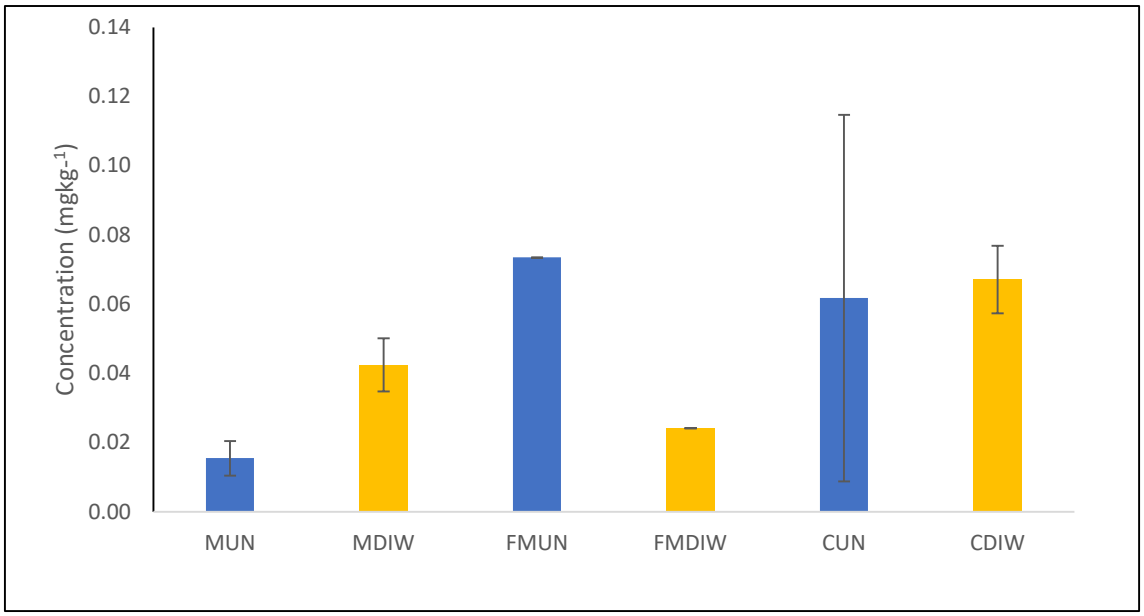

Fig.4.5 :A comparison of the effect of unwashed and washed treatments on the Cd analysis of different hair samples.

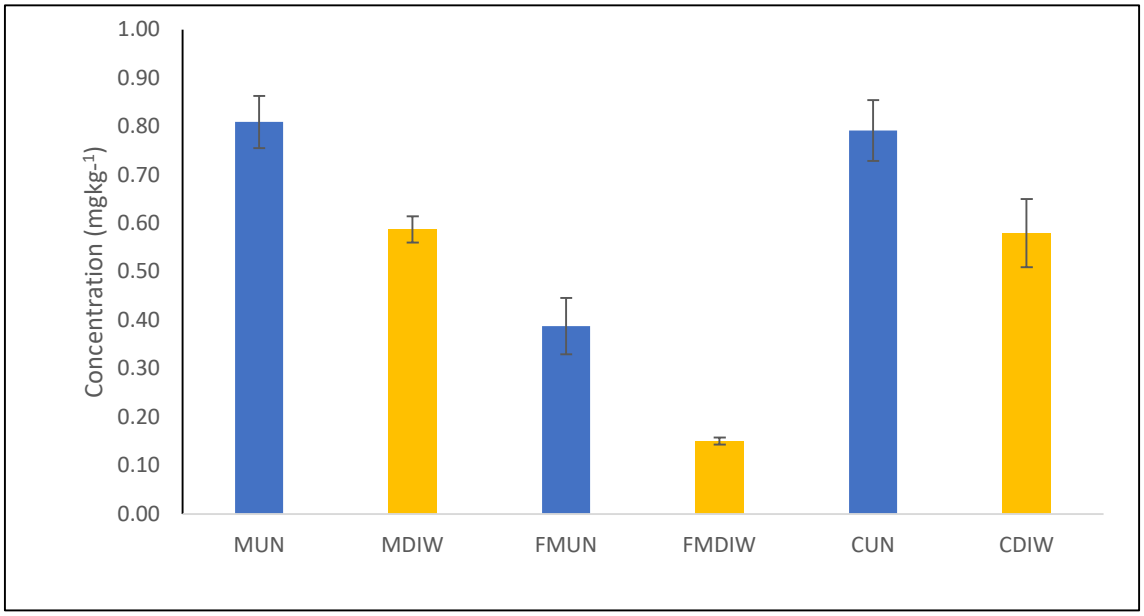

Fig.4.6 :A comparison of the effect of unwashed and washed treatments on the Cr analysis of different hair samples.

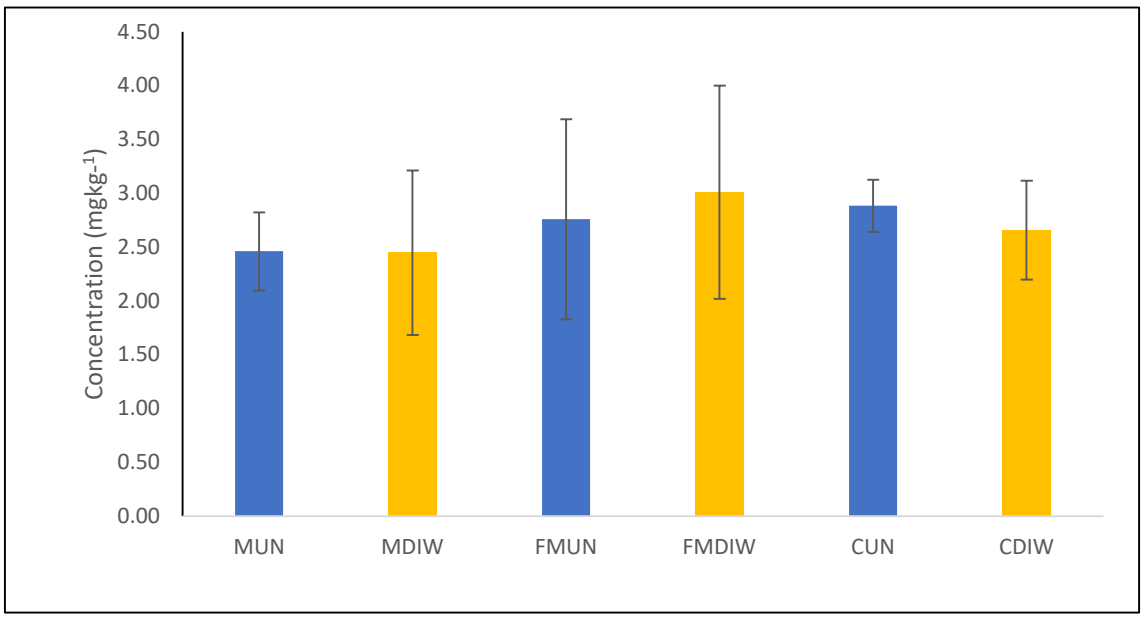

Fig.4.7 :A comparison of the effect of unwashed and washed treatments on the Mn analysis of different hair samples.

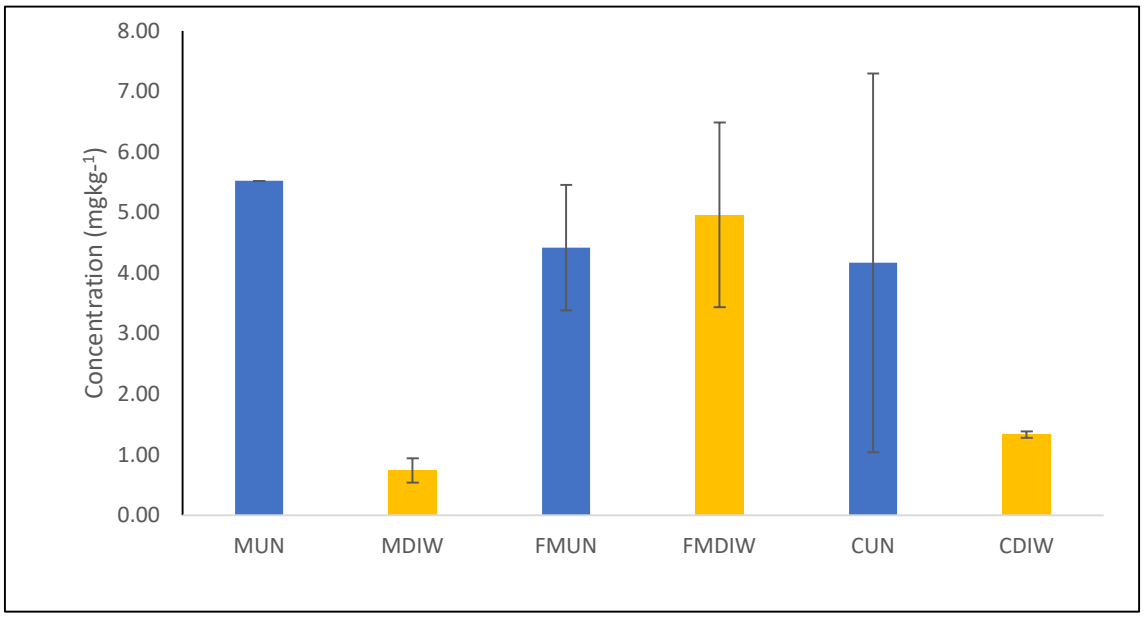

Fig.4.8 :A comparison of the effect of unwashed and washed treatments on the Ni analysis of different hair samples.

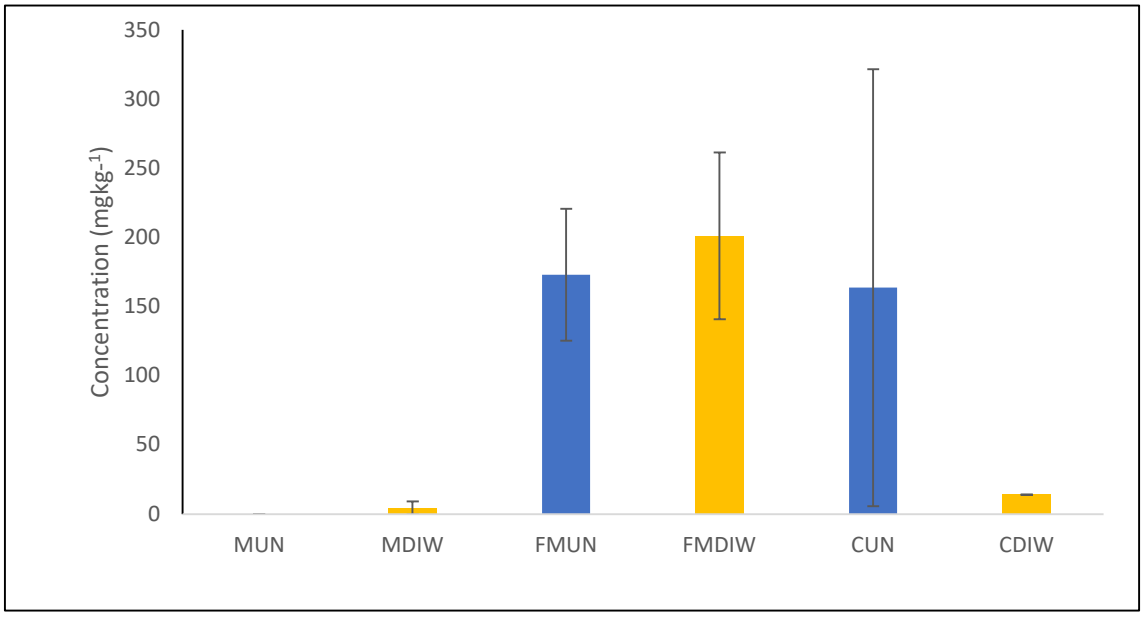

Fig.4.9 :A comparison of the effect of unwashed and washed treatments on the Pb analysis of different hair samples.

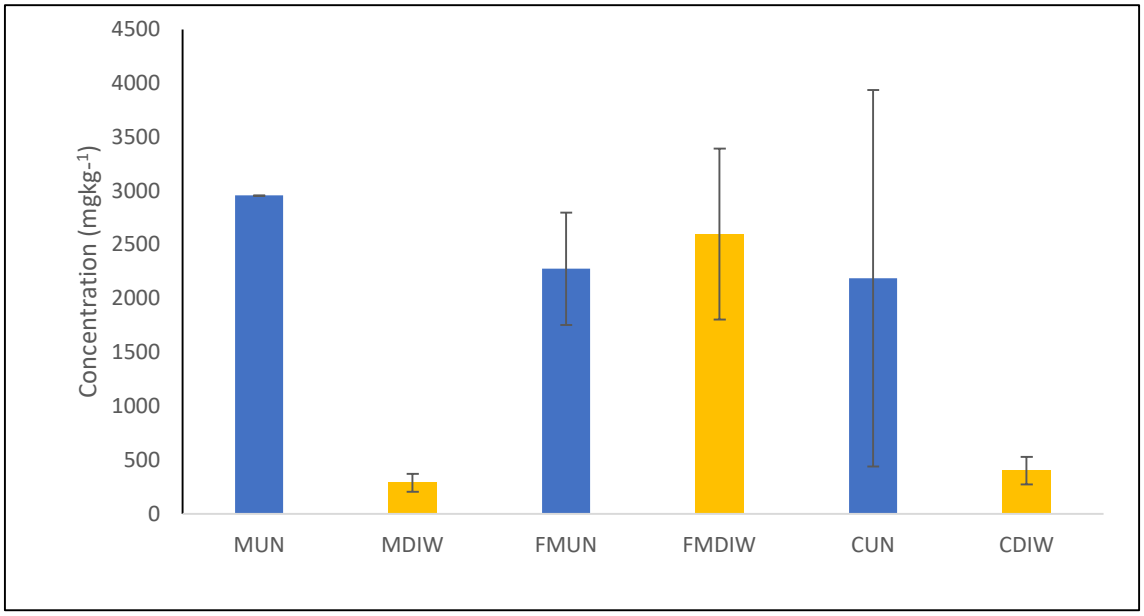

Fig.4.10 :A comparison of the effect of unwashed and washed treatments on the Zn analysis of different hair samples.

The data obtained by the second phase pre-treatment procedures were sometimes subject to large variation and where no error bars are presented, there were not three values that gave any repeatability. In general, though, Cr, Mn, Ni, Pb and Zn data for the female samples showed no significant differences between the two wash methods. For both composite and male samples there were larger variations between the two methods, except for Mn which gave very similar results for both methods.

The male samples were in general low in Pb and the female samples much higher. It is unclear why the DIW Pb results for the composite sample is so low. The Cr data were the exact opposite, displaying higher values for males than females.

These results were confirmed by the application of a one way ANOVA comparing the different results for elements, while the t-test was applied to unwashed and washed results.

The variation in results obtained in this phase of the investigation, corresponds to the variances reported between previously recorded studies discussed in paragraph 2.1.7. The variation in absolute values between phase 1 and phase 2 of the pre-treatment optimisation is not clear. It could be that the samples have dried out and degraded significantly during the 2 years that it was left in the cupboard. On the other hand, the data reported in Chapter five of this study, is generally also in the ppm range rather than the ppb range. Therefore, it is believed that the latter methods of pre-treatment are the most reliable and produced trustworthy results.

Based on the results shown above, the large variations observed with Triton and acetone washes, and the possibility of external contamination in the unwashed samples, it has been decided to eliminate further analyses of unwashed hair and continue with Millipore water washed hair in the rest of the work. This portion of the work concludes the objective of this investigation to compare different hair washing methods so that the most reliable method for the rest of the hair analysis process could be established.

5 Results and Discussion of the Case Study

5.1 Metal Impact on Population of Greater Manchester

One of the important objectives of this investigation was to determine whether the selected potentially toxic metal profiles of the male and female hair samples could be correlated to the official death rates in nine GM boroughs. These results include qualitative and quantitative analyses of the inorganic metal content of hair samples from male and female members of the population.

The results obtained are presented in the following figures. Independent sample T-tests were conducted for gender type versus the measured elements and location versus metal element values (Appendices-F:2).

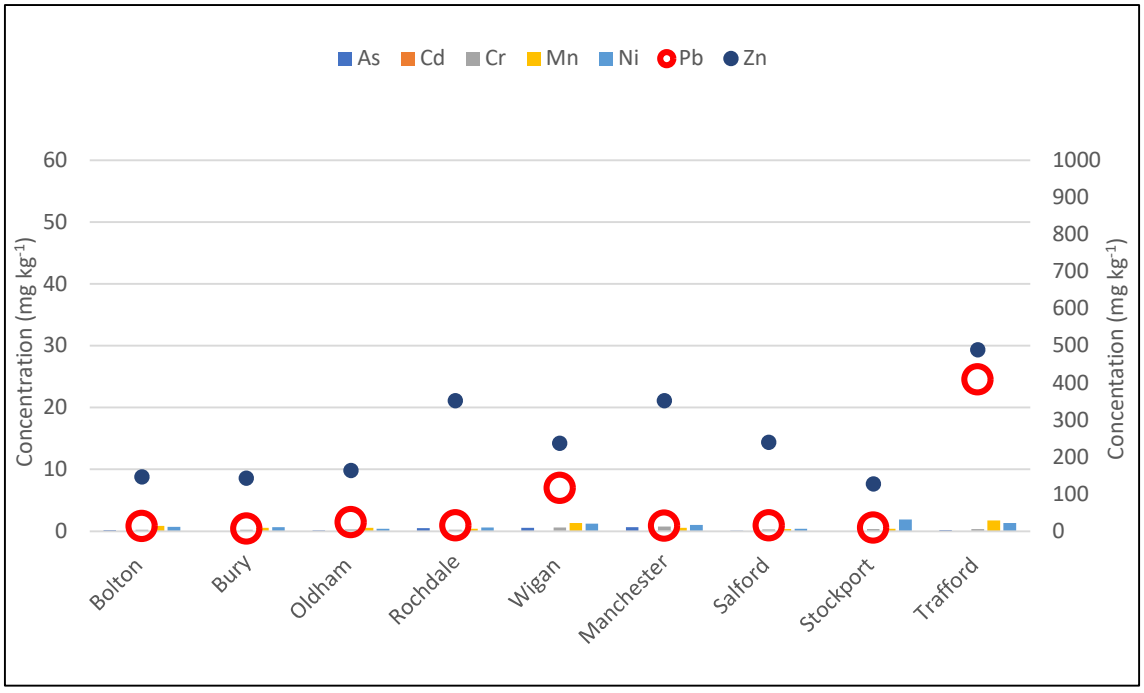

Fig.5.1 :Possible toxic metal element contents in female hair collected in Greater Manchester regions with high and low mortality rates. Pb is indicated as a circle although the primary vertical axis represent its concentration, to distinguish it from the other element. Zn is indicated on the secondary (right hand) axis.

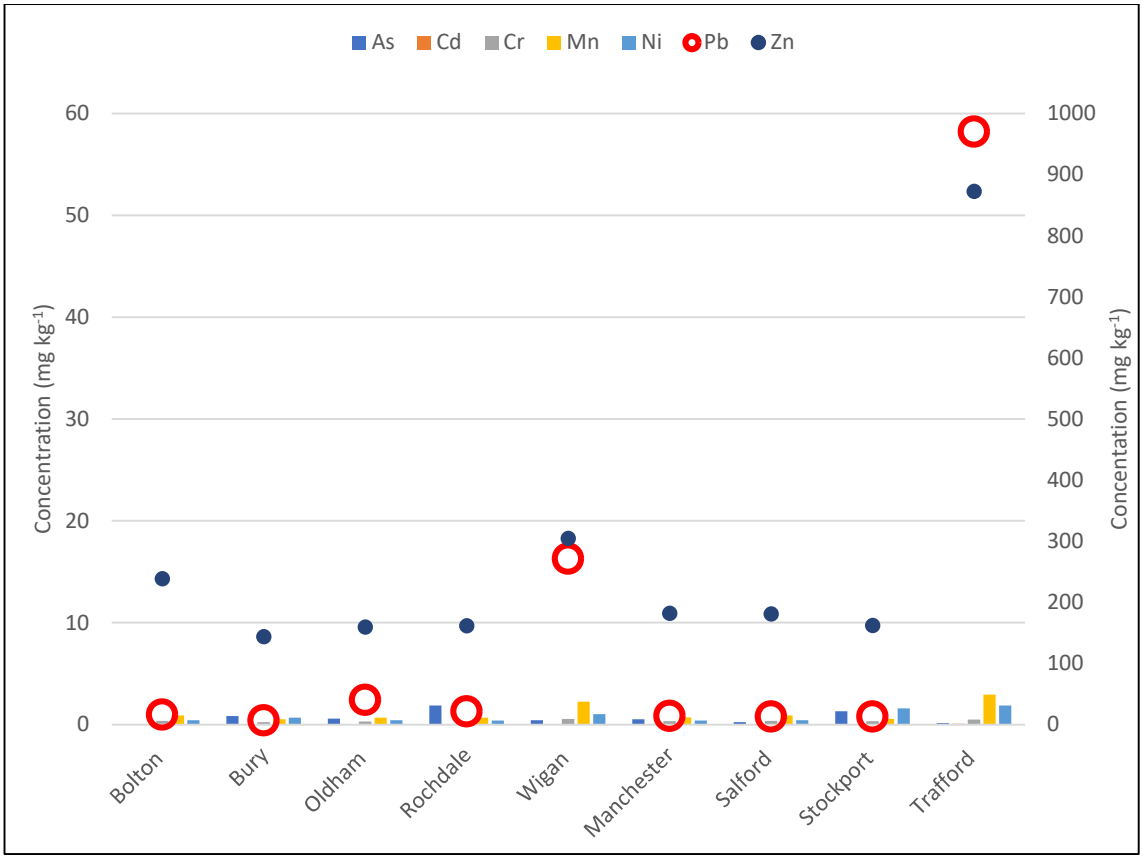

Fig.5.2 :Possible toxic metal elements contents in male hair collected in Greater Manchester regions with high and low mortality rates. Pb is indicated as a circle although the primary vertical axis represent its concentration, to distinguish it from the other element. Zn is indicated on the secondary (right hand) axis.

Fig.5.1 illustrates that the Zn and Pb concentrations level in the female hair was higher than all of the other metallic elements measured, while the highest Pb (24 mg kg^{-1}) and Zn (489 mg kg^{-1}) concentrations were recorded in the borough of Trafford. These values correlate well with a study by Rehman et al. (2018) reporting 46 and 558 $mgkg^{-1}$, respectively for hair collected from Pakistanis. In the other hand it is much higher than those reported in Gangzou, China (Huang et al., 2014). A similar pattern can be distinguished in the male hair samples analysed and plotted in Fig.5.2. From Figs.5.1 and 5.2 it can be seen that the boroughs of Wigan and Trafford displayed elevated Zn and Pb concentrations in the male and female hair samples compared to the rest of the areas in GM. There was also a good correlation between the Zn and Pb values; i.e., the hair samples from Wigan and Trafford, with the highest Zn values, also had the highest Pb concentrations. However, it is noticed that the Trafford male samples had Pb and Zn levels that were two times higher than the female samples. The rest of the metal levels are mostly present in high ppb levels.

There are obvious differences between the boroughs, which has also been noted by Rehman et al. (2018) who also report on various other publications on potentially toxic elements in hair. It is clear from that survey that concentrations can vary significantly from one location to another. In addition to the differences observed between boroughs and between the different metals, we also observed differences in the variance of the data. Figs.5.3–5.8 make use of whisker plots to illustrate the 25 and 75% quartiles, as well as the median and average of the data. A reasonably high variation was observed in the values obtained for the Zn and Pb analyses (large standard variation on the average value), which is probably due to a particular sample which was not homogenised as well as others. Figs.5.3–5.8 highlight just how large these variances are in the Pb and Zn analyses of male and female hair samples collected from different ares. Care should therefore be exercised in the interpretation of the results and attaching too much value to it. In view of this, it may be useful to have follow-up studies in the Trafford and Wigan areas and collect more samples to be analysed.

Fig.5.3 :Whisker plot, indicating the median and the average as a cross, of Female hair Zn data comparison between selected areas of Greater Manchester.

Fig.5.4 :Whisker plot, indicating the median and the average as a cross, of Male hair Zn data comparison between selected areas of Greater Manchester.

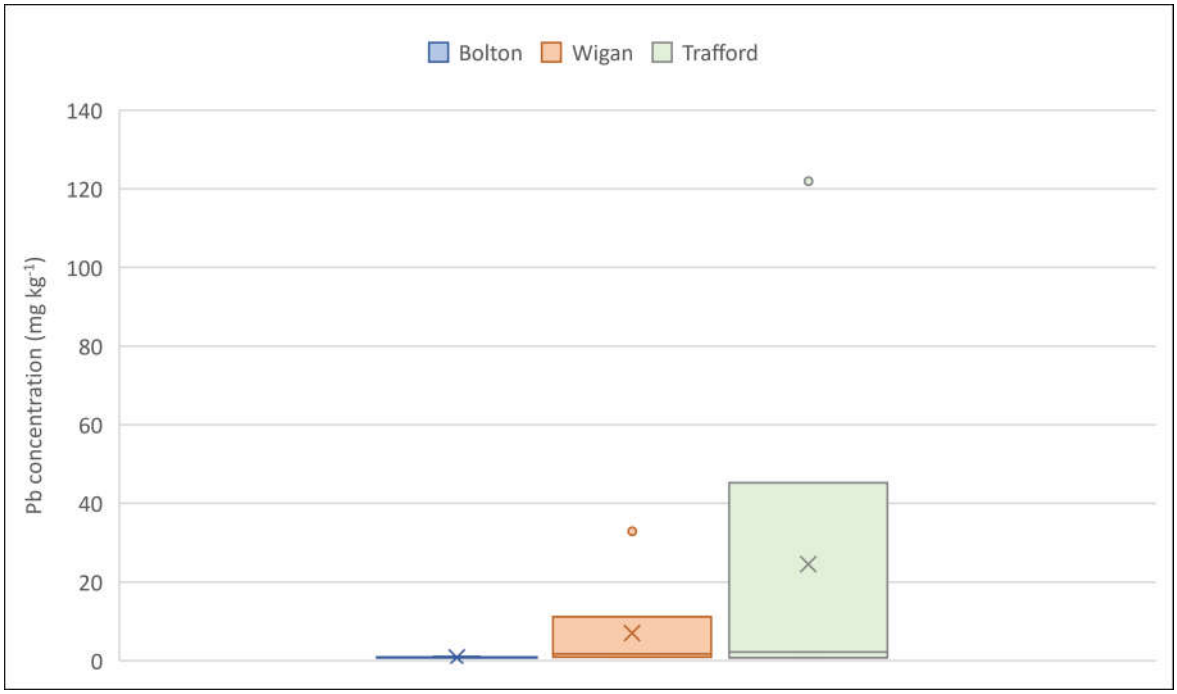

Fig.5.5 :Whisker plot, indicating the median and the average as a cross, of Female hair Pb data comparison between selected areas of Greater Manchester.

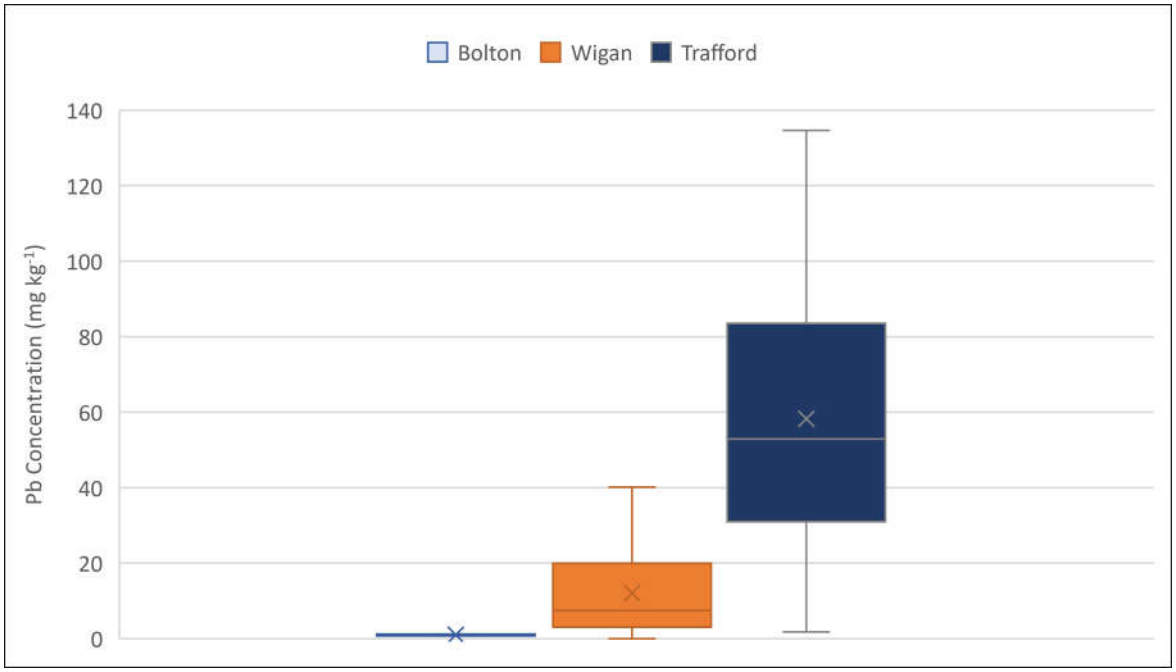

Fig.5.6 :Whisker plot, indicating the median and the average as a cross, of Male hair Pb data comparison between selected areas of Greater Manchester.

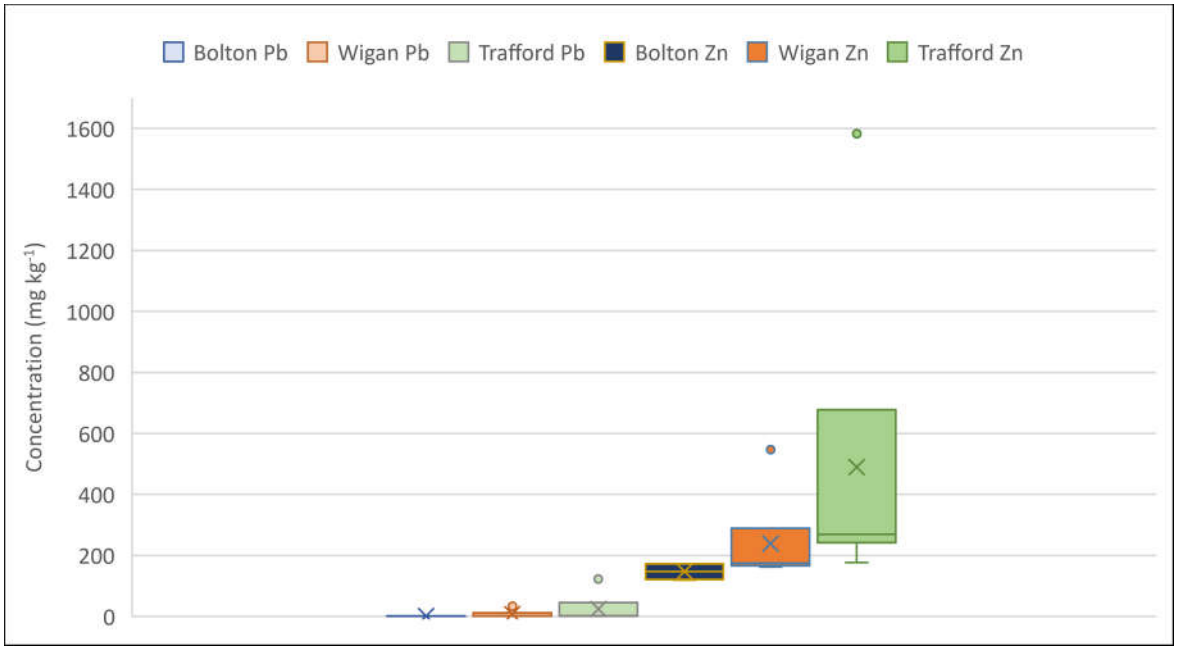

Fig.5.7 :Whisker plot, indicating the median and the average as a cross, of Female hair Pb and Zn comparison between selected areas of Greater Manchester.

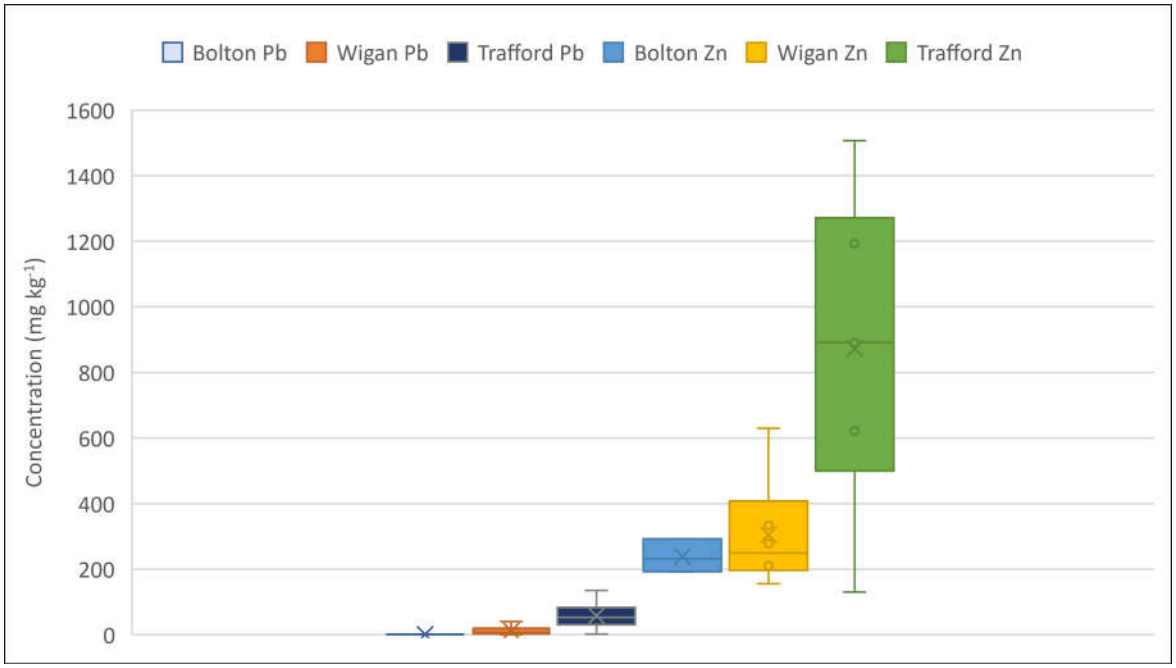

Fig.5.8 :Whisker plot, indicating the median and the average as a cross, of Male hair Pb and Zn data comparison between selected areas of Greater Manchester.

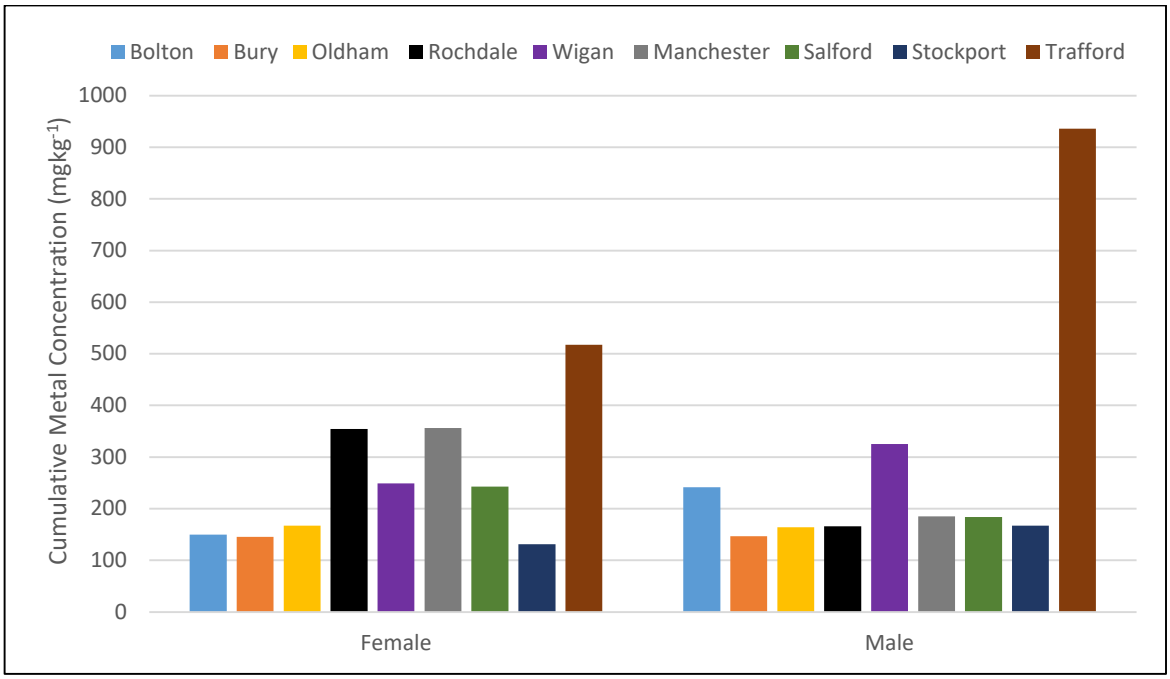

Fig.5.9 :Cumulative representation of female and male hair sample metal contents in the various areas of Greater Manchester.

The results summarised in Fig.5.9, indicate that the cumulative metal contents in the female and male hair is the highest overall in Trafford. The three boroughs with the highest cumulative amounts of metals in the female hair samples are Manchester, Rochdale and Trafford, while for the male hair samples, they are Bolton, Wigan and Trafford. This is contrary to the expectation from the findings in Purdam's (2016) study, which indicated that the life expectancy of males and females in Trafford are higher than in Rochdale. While these results are preliminary in nature, it would be worthwhile to pursue more detailed studies in Trafford, as male and female hair samples indicate a higher metal content than the other areas in GM.

In Table 1 as well as Figs.1.1-1.8 in the introduction section, Bury is confirmed as having the highest mortality rate of the GM boroughs. The results in Fig.5.9 reflect that Trafford is the borough with the highest metal content in Greater Manchester for both male and female populations whilst having one of the lowest mortality rates. The variability between male and female death rates reported by Purdam (2016) and Patel (2017) in different years during the period 2014–2017 makes a comparison with the results from this study impossible. This is justification for much larger and more comprehensive studies with a narrower focus than was the case here. For example, it would be interesting to investigate whether the high levels of Pb and Zn found in all of the subjects in Trafford may be linked to certain conditions, such as: ASD or ADHD, as reported by Grandjean and Landrigan (2006), Al-Farsi et al. (2013) and Yasuda et al. (2013); obesity, cancer and BMI, as reported by Brown et al. (2018) and Hooper et al. (2018); or increased blood pressure in adults, as reported by the WHO Convention Task Force.

While the study by Benach et al. (2004) in the Huelva province in Spain found a clear link between environmental pollution as a result of metal contamination and the local population's health, it did not include mortality rates and hence the current investigation's aim of establishing such a link between metal concentration and death rate could not be proven with the limited data available. Table 2.1 did however found much higher heavy metal contents in the hair samples used than those reported in the literature for Ni, Pb, Cu, Fe and Zn which may hint towards a link with the higher mortality rates in general in the Northwest compared to the southern (generally more affluent) counties in the UK.

5.2 POPs Impact on Population of Greater Manchester

Some of the PCBs, like PCB180, PCB28, PCB138 and PAHs, like Fluoranthene, Pyrene, Benz(a)anthracene, Benzo(g,h,i) Perylene, Chrysene, Indeno(1,2,3), were below the limit of detection or absent from 90% of the results (see Appendices-G). For these reasons these POPs compounds were eliminated from the final analysis of the hair samples in the nine areas investigated.

In the female hair samples (Fig.5.10), the different PCB concentrations, especially PCB118, are the highest in the borough of Bury, which is one of the areas with a high mortality rate. Among the southern areas in the city (classified as lower mortality rate), the different PCB concentrations are the highest in Stockport (one should take cognisance of the fact that Stockport has the highest mortality rate of the southern boroughs). Qiao et al. (2019) investigated hair samples taken from the scalp at 3 cm distances for its organic contaminants. They reported significantly lower values than what we report in this study and indicated that the most abundant PCB was 28 and not 118 as we report here. Qiao et al. (2019) reported that there was a linear increase in PCB concentration from proximal to distal distances and that the “aged” hair seems to be more prone to external sorption of PCBs. To that end, it is then not surprising that this study showed much higher values, since these would most probably be hair trimmings from medium to long hair. In addition, the hair would also be quite exposed to the environment before being collected.

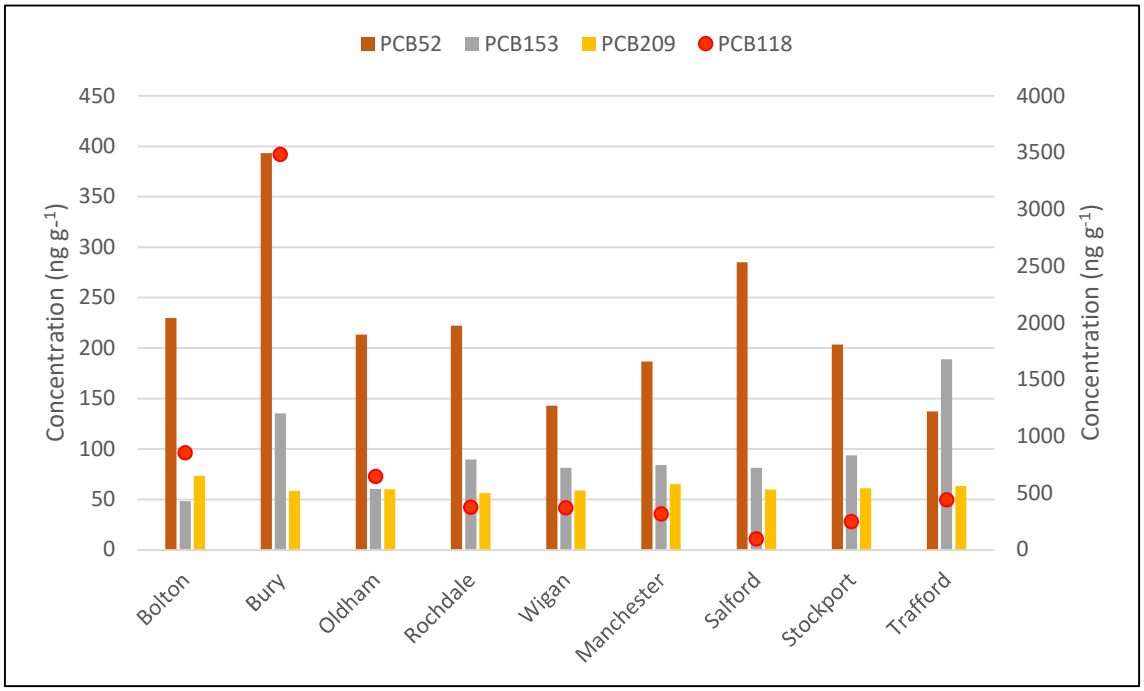

Fig.5.10 :PCBs in female hair collected in Greater Manchester within the regions with high and low mortality rates. PCB118 is on the secondary (right hand) axis.

Among the male hair samples (Fig.5.11), there is not much difference between the concentration levels of PBC-52, -153 and -209 in comparison with the female samples. PCB-118, although also significantly higher than the other PCBs in most cases, it is not as high as in the female samples. Where Bury had the highest PCB-118 level for female hair it is now the lowest in the male samples. Even though PCB-118 is lower in concentration for the male samples it is still an order of a magnitude higher than the values reported by Qiao et al. (2019).

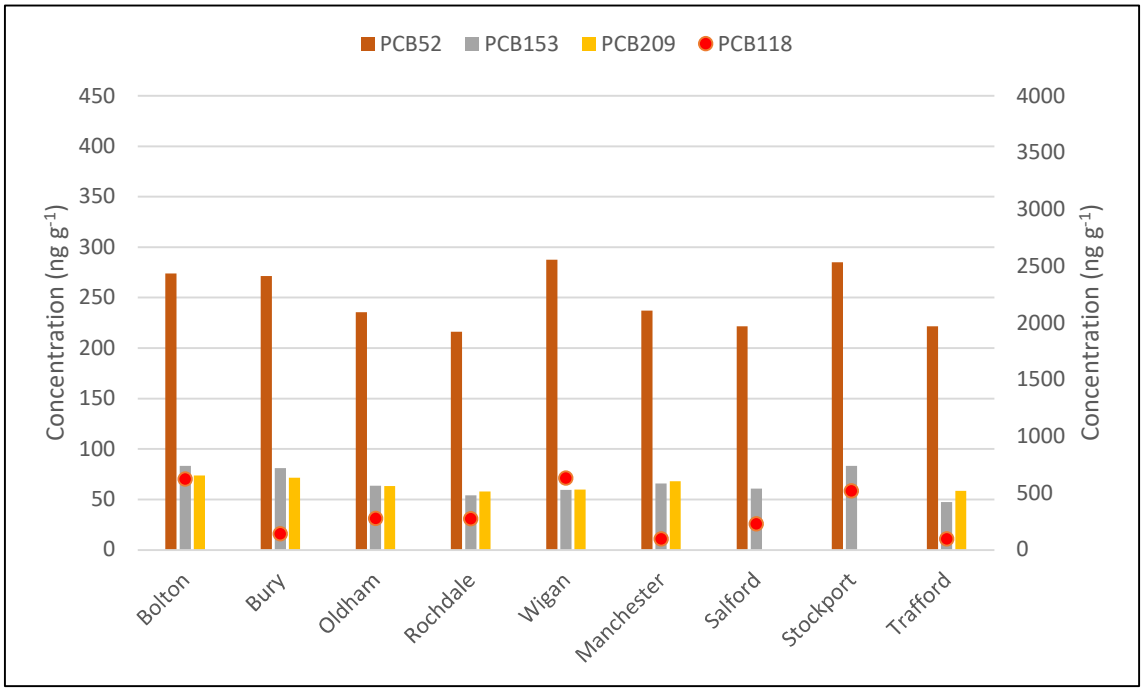

Fig.5.11 :PCBs in male hair collected in Greater Manchester regions with high and low mortality rates. PCB118 is on the secondary (right hand) axis.

A closer look at the different PCB contents of male and female hair, reveals some important observations (Fig.5.12). Firstly, apart from PCB118, the rest of the PCBs have a much lower concentration and are not too dissimilar in their occurrence. Secondly, the three PCBs other than PCB118, had similar concentrations compared to each other in both male and female hair samples. Lastly, PCB 118 is clearly present in much higher concentrations than the rest in both female and male hair samples, and also is present to a much larger extent in female hair samples than in male hair samples. The reason why PCB118 in female hair was higher than in male hair needs to be investigated further, as well as the possible influence (mostly) female hair colouring could have in this regard.

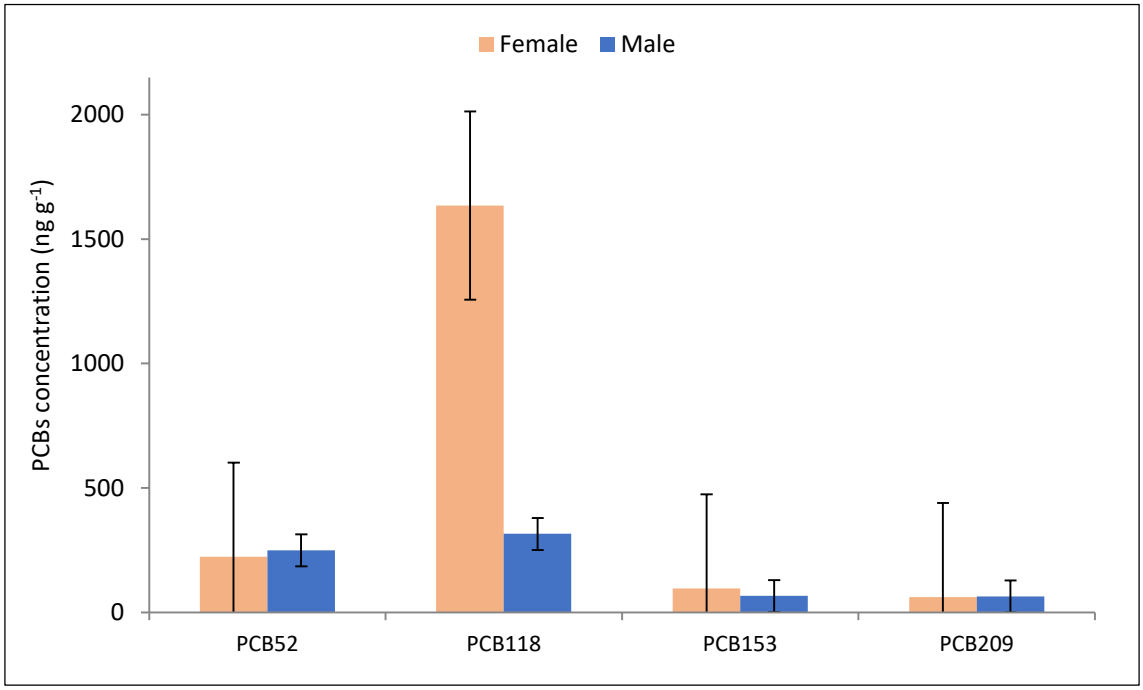

Fig.5.12 :A total (average) of the female and male hair sample PCBs content for all samples collected in Greater Manchester.

The cumulative concentrations of all the PCB in both female and male hair samples confirm the observations already depicted in the previous graphs (Fig.5.13). In general, the female hair samples in all the boroughs displayed higher cumulative PCB concentrations than their male counterparts from the same areas, except for Wigan and Stockport. The total concentration is also much higher for the 4 PCBs if compare to the data from the Qiao et al. (2019) paper, which reports a total of 86.5 ng g^{-1} for the 4 PCBs. Again, this difference should be further investigated, but one of the reasons could be the aging of the samples and external factors. There also a discernible trend, especially in the female hair group, that the northern and western boroughs in GM have higher cumulative PCB concentrations than in the central and southern boroughs. While there is existing literature on the occurrence and effects of PCBs in the environment, as discussed in Section 1.2.2, no reports could be found of a study in Manchester to compare the values obtained in this investigation with. A clear link to the mortality rates, except for PCB118 in Bury, could also not be found among the rest of the analysed samples.

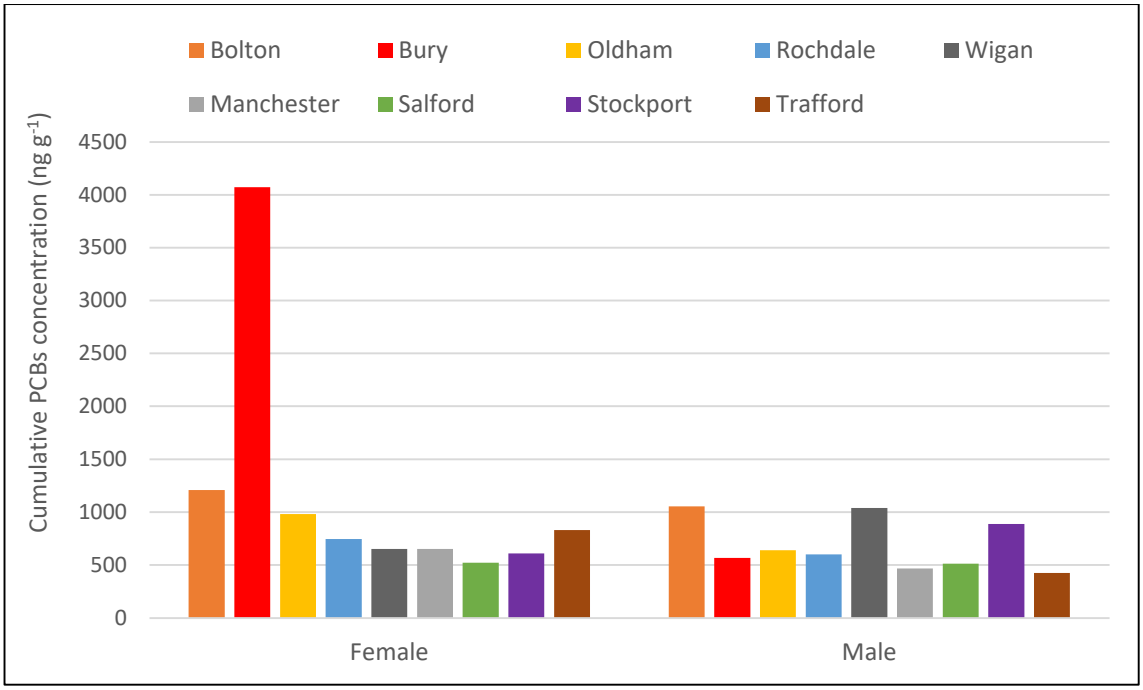

Fig.5.13 :Cumulative representation of female and male hair sample PCBs in various areas of Greater Manchester.

The PAH concentrations are given in Figs.5.14–5.16. The PAH data are mainly discussed in a qualitative way as LODs on method blanks could not be performed and therefore, the QUeChERs method for extraction and other factors may lead to large errors. Firstly, we observe that acenaphthene is present in much higher abundance as any of the other PAHs, with Stockport showing the highest value (again remember that the death rate in Stockport is towards the high end, albeit in the sout), similar to the PCB-118 profile. Secondly, apart from anthracene being high in Bolton and some extent Wigan (potentially outliers) the rest of the PAHs are similar in concentration levels across the boroughs and significantly lower than the acenaphthene. These unusually high values bear resemblance to the high metal values found in hair samples at Wigan.

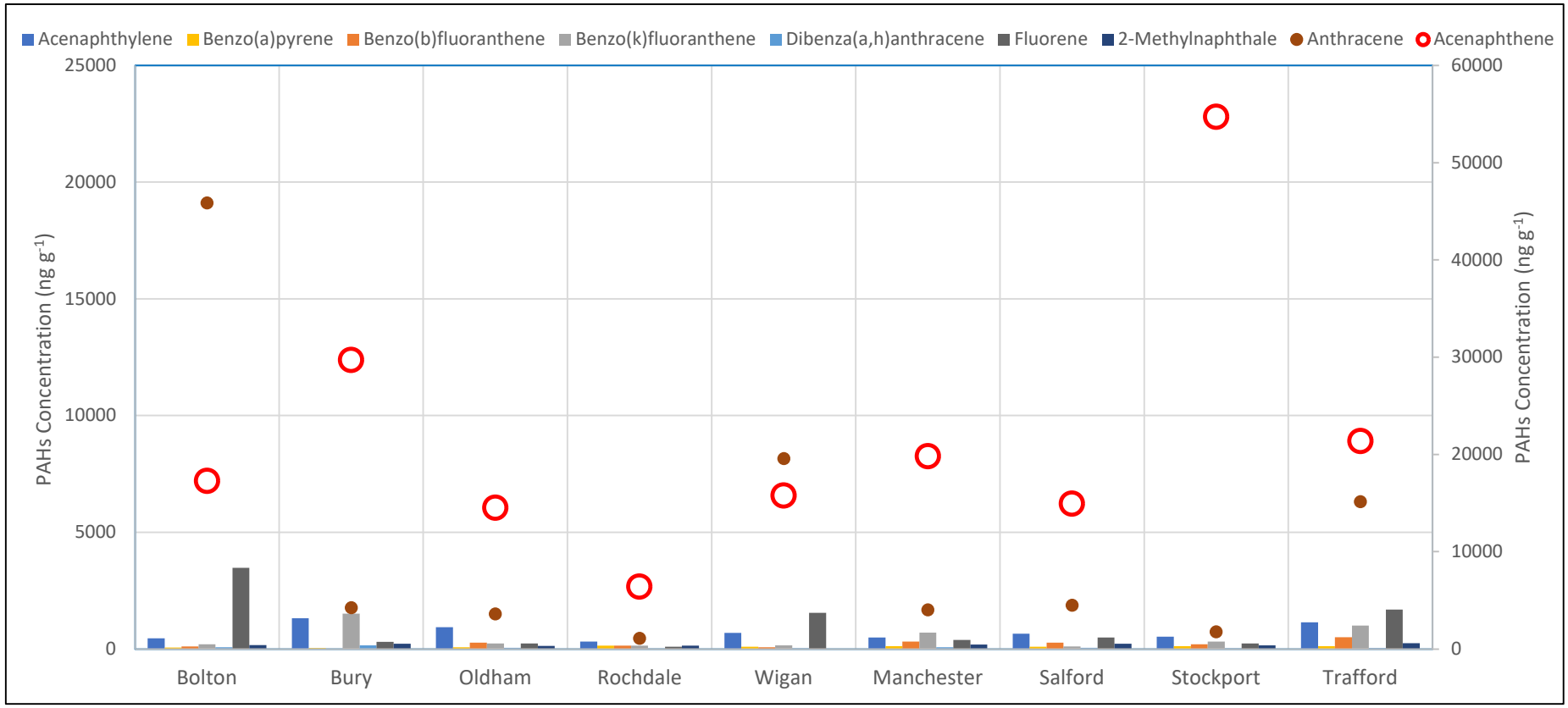

Fig.5.14 :PAHs compound contents in female hair collected in Greater Manchester regions with high and low mortality rates. Acenaphthene is indicated on the right-hand axis.

Fig.5.15 displays the PAH profile in males' hair, showing raised levels of acenaphthene and naphthalene. Anthracene is also slightly elevated in Bolton, but the rest of the PAH levels seem to be similar to the female profile.

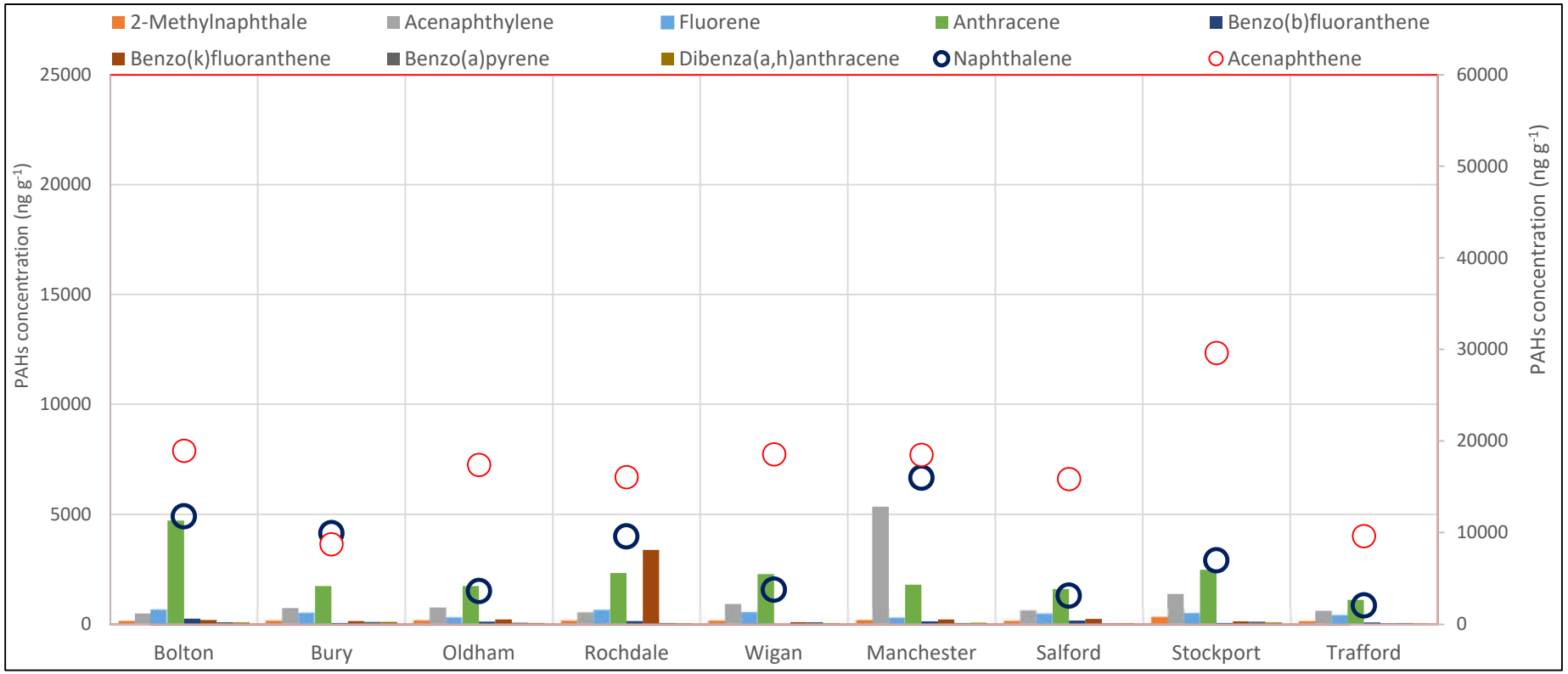

Fig.5.15 :PAHs compound contents in male hair collected in Greater Manchester regions with high and low mortality rates. Acenaphthene and naphthalene are indicated on the right-hand axis.

Fig.5.16 shows a comparison of average PAHs across the boroughs for male and female hair. It is clear that the PAH contents are dominated by three compounds, i.e. acenaphthene, anthracene and naphthalene (see Fig. 5.16). The acenaphthene has much higher levels than the others followed by naphthalene. Palazzi et al. (2018) reported highest concentrations for phenanthrene, followed by fluoranthene, pyrene and fluorine, which in our case were all well below the LOD. Palazzi et al. (2018) collected hair from Chinese women in two different cities with different air quality indices. Apart from the use of the QUeChERs extraction technique, which could have made a difference to the results obtained, these differences could also be ascribed to the significantly different environment (therefore exposure) and sampling methodology followed by us and the authors of this paper.

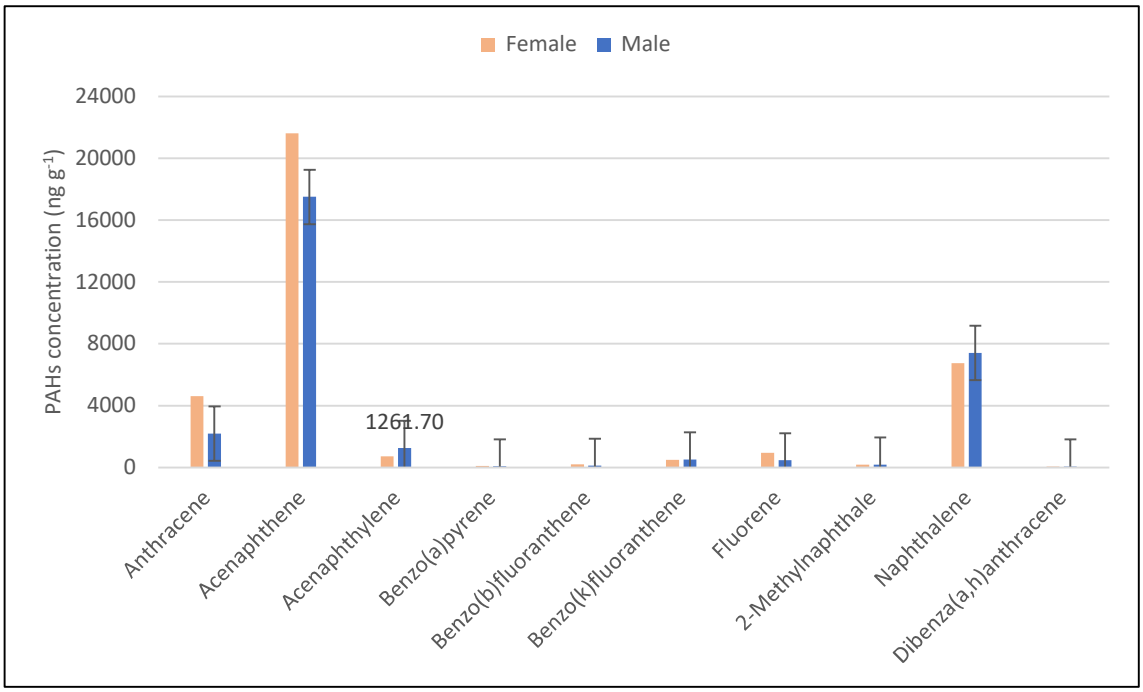

Fig.5.16 :Comparison of PAHs concentration in female and male hair in Greater Manchester population.

In the light of the above discussion, the data will rather be discussed qualitatively as a sum of the PAHs detected. Figure 5.17 displays the sum of PAH across the different boroughs.

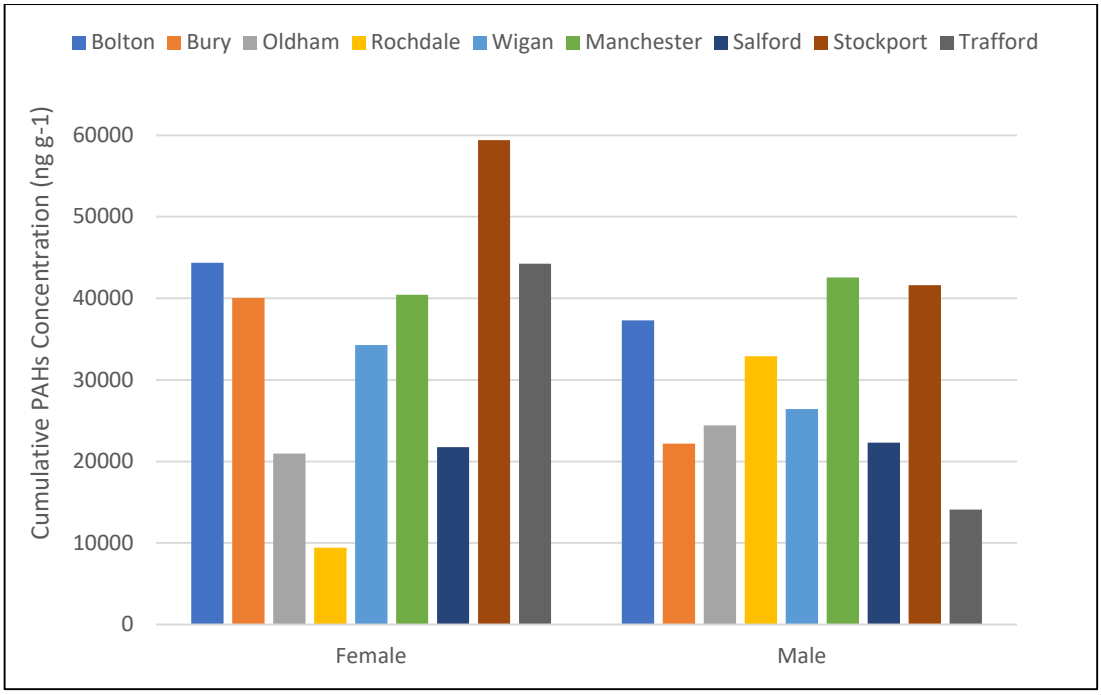

Fig.5.17 :Cumulative representation of female and male hair sample PAHs in various areas of Greater Manchester.

Fig.5.17 provides an overview of the areas investigated. Among the nine different areas investigated, Bolton, Manchester and Stockport stand out as the areas with the highest total concentrations. For female hair, Bury and Trafford also showed similar cumulative concentrations. This is not entirely the same profile from what was observed in the case of high metal contents in the hair, although Bolton appeared in both cases to have high metal and PAHs values. Combined Stockport seems to have the highest PAHs concentration, while for PCBs it was Bury. Stockport data did not display a high metal concentration in male and female hair samples, but although it is categorised as a lower mortality rate than Bolton, Bury, Oldham, Rochdale and Wigan, it is not much lower than the rate in these boroughs. Bolton on the other hand, while not having such a high metal concentrations as Bury, also had reasonable metal levels in male and female hair and is among the boroughs with a higher mortality rate.

In an effort to compare the total toxic burden in the hair samples, cumulative POP and metal levels were determined, by simply summing the different components. Figure 5.18 provides an overview of the emerging toxic profile.

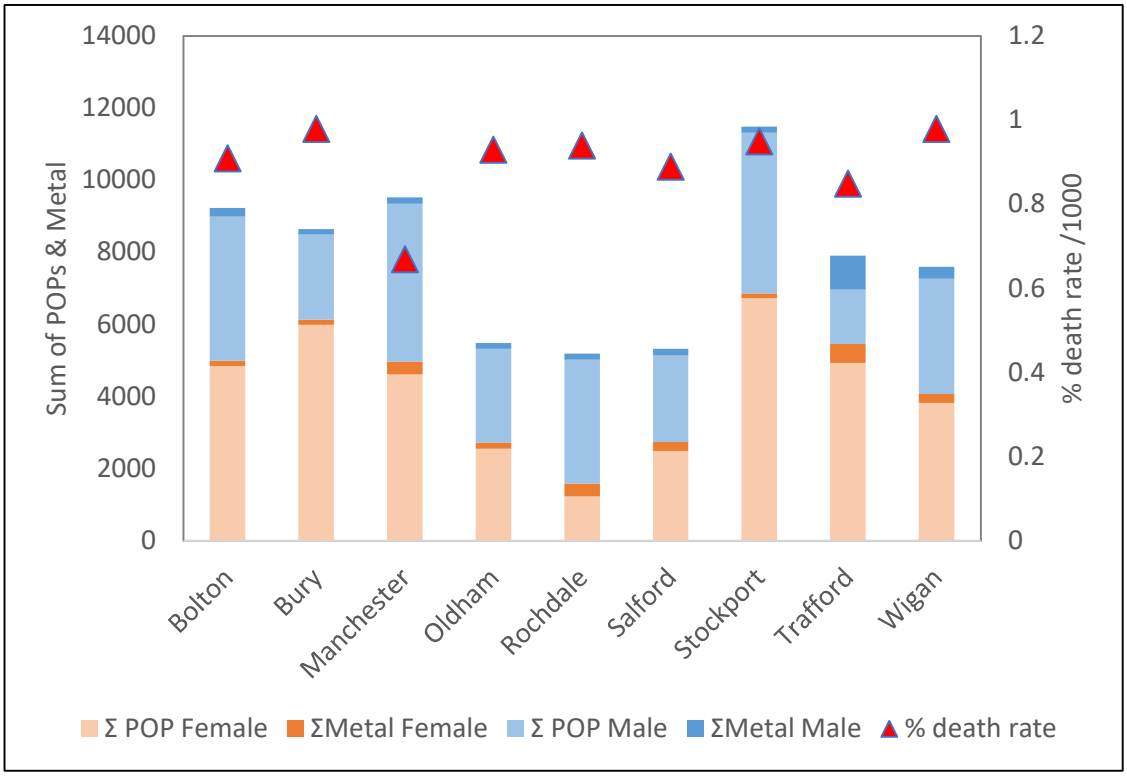

Fig.5.18 :Cumulative POP and metal concentration levels for male and female hair samples taken from the 9 boroughs in GM compared with percentage death rates.

It is clear that neither the male nor the female total toxic budget of the hair samples resembles the same profile than the overall death rates as published online. There also does not seem to be a direct relationship between the overall concentration and death rate. Stockport had the highest total concentration and is nearly on par with the high mortality rates of Wigan and Bury. On the other hand, Manchester had the second highest total concentration but has the lowest mortality rate of all the boroughs investigated. It seems that the data presented in this study showed that hair could not be used as a reliable biomarker for environmental pollution and other stressors if it was collected and analysed in the way we described here.

6. Conclusions and Recommendations

At the start of this investigation, the aim was to obtain a fingerprint of metal concentrations and selected POPs in male and female hair clippings collected from barbers and hairdressers across GM. These data were then correlated with mortality data, obtained from governmental websites, to establish whether any links between the two sets exist or any specific trends could be identified.

To accomplish this aim, the following objectives were set:

Collect hair from nine different areas, half of which have a low mortality rate and half a higher mortality rate, and analyse the hair samples.

According to the official death rate statistical data (discussed in Chapter 1), the boroughs of Bolton, Bury, Oldham, Rochdale and Wigan, located mostly in the northern and western side of Greater Manchester, have been identified as areas with higher death rates and lower life expectancies than the rest. The boroughs of Manchester, Trafford, Stockport and Salford, located in the central and southern part of Greater Manchester were found to have some of the lowest death rates and highest life expectancies. These areas were therefore chosen for the sample collection. In each one of these boroughs, two barbershops for the collection of male hair samples and two hairdresser shops for the collection of female hair samples were chosen and the collected hair samples of each type were combined in a composite sample for the area before the analysis was performed.

Compare different hair washing methods and verify the most reliable method for the rest of the hair sampling process.

In the next phase of the investigation, different methods and procedures were investigated to determine whether or not the hair should be washed prior to the analysis. Again, the literature indicated potential ways in which this could be accomplished, e.g. by using combinations of water and acetone in different sequences, or employing Triton X100. There was no consensus in the reported literature regarding the optimum approach to apply, or the one that would produce the most consistent results. After trying the various

different washing methodologies, it was found that hair washed with Triton X100 consistently yielded different analysed values for metal contents compared to the other washing combinations used. Finally, it was decided to compare the metal values measured from hair washed with only deionised water and non-washed hair with each other, before a simple wash with deionised water only was selected as the method of choice for this investigation.

Optimised the full digestion method for potentially toxic elemental determinations by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and Gas Chromatography-Mass Spectrometry (GC-MS) for POPs determinations.

A microwave digestion method was selected to dissolve the hair samples completely before the solutions were analysed for selected metal elements and various PAHs and PCBs POPS compounds. After some trial and error variations regarding power, time, and acid volumes, an optimised procedure was developed for complete dissolution and applied in this investigation to prepare the samples appropriately for analyses. Optimised instrumental settings were derived with the help of experienced technicians and instrumental manuals. The QUECHERs method was used for the GC sample preparation due to its quick and easy way of extracting organic compounds. Very little optimisation could have been implemented within the scope of this research project and this is one of the areas that would need to be further optimised in the future.

Identify, qualitatively and quantitatively, the presence of selected potentially toxic metallic elements and POP's in the hair using ICP-OES and GC-MS, respectively.

Data obtained by ICP-OES and GC-MS analyses were correlated with the calibration curves for the ICP-OES and standard libraries of the compounds for the GC-MS identification of various PCBs and PAHs. Standard statistical tests were applied to check the validity of the measured values obtained, and these are summarised in the attached appendices to this thesis.

For the ICP measurements, the LODs and LOQs were determined for each metallic element in the usual way. Despite repeated analyses, large variations in the standard deviations were sometimes encountered in the metallic elemental analyses of hair samples from various GM areas. It was clear that the homogenisation of the combined hair samples was, at times, dissatisfactory. Zn and Pb were the two metallic elements present in the largest concentrations in both the male and female hair samples in all the boroughs investigated. In addition, the Zn and Pb values were up to two orders of magnitude higher than the rest of the metallic elements measured, i.e. Mn, Ni, Cd and Cr (similar to the findings of Rehman et al., 2018). There were wide variations in the individual metallic elements analysed, as well as in the cumulative metallic analyses for all of the metals in a single male/female hair sample. The boroughs with the lowest and highest metallic elemental concentrations occurred in the male and female hair could be easily identified, and the Pb and Zn contents were always found to be the lowest in Bolton and the highest in Trafford, regardless of the sex of the samples. The cumulative metal contents in the female and male hair were the highest overall in Trafford. The three boroughs with the highest cumulative amounts of metals in the female hair samples were Manchester, Rochdale and Trafford, while for the male hair samples it was Bolton, Wigan and Trafford.

With regards to the PCB contents in the male and female hair, PCB 118 is clearly present in far higher concentrations than the rest in both the female and male hair samples, and also present to a far larger extent in the female hair samples than in the male hair samples. The rest of the PCBs have a far lower concentration and are relatively similar in occurrence. Secondly, the three PCBs, apart from PCB118, had similar concentrations in both the male and female hair samples.

The PAH analyses revealed that the PAH contents are dominated by these three compounds, e.g. Acenaphthene, Anthracene and Naphthalene. In the female hair samples, high values of Acenaphthene were found in Bury and Stockport, which is similar to the finding for PCb118. Unusually high values compared to the rest of the boroughs were found for Anthracene in Bolton and Wigan. In the male hair samples, Acenaphthene was also found in higher concentrations than any of the other PAHs, while Naphthalene occurred in

higher concentrations in the male hair samples than the rest of the PAHs, but was generally at a lower level than Acenaphthene. The profiles of the rest of the PAHS analysed were relatively different between the sexes.

Correlate the presence of selected potentially toxic elements and organic compounds with the mortality rate statistics in the collection areas.

Sadly, despite the expectations at the start of this study, no correlation was found between the death rates and/or life expectancy of the populations in the various boroughs of GM and the metallic element concentration profiles and POPs contents of the male and female hair samples, as is illustrated in Fig.5.18. in fact, Trafford, which has one of the highest life expectancies for males and females, and is historically fairly industrialised, has the highest Pb and Zn concentrations in the male and female hair samples from all the boroughs investigated, despite Pb being generally considered to be an accumulative toxic element for human health.

Despite the bold hypotheses made at the start of the investigation, namely that there is a correlation between the amount of a certain element and/or POPs and mortality rate in a specific metropolitan area, and that these differences in concentrations within female and male hair correlate with the mortality rate for females and males in that area, the findings of this investigation fail to support these. This may be due to a number of different reasons. Palazzi et al. (2019) report that the biomonitoring of children's exposure to urban pollution by the analysis of POPs provided compelling data linking exposure with POP levels. The biggest difference between the data and methodology followed in our study is that the hair was collected from selected individuals by Palazzi's team, from whom full details about their respective exposure were available. It is believed that, when adopting our approach, the cohort size needs to be increased and anonymous questionnaires completed to ensure better prior knowledge of the sample. In addition, a far larger investigation to establish the potential differences within each borough, and including more boroughs in the investigation, would provide a more complete picture for comparison purposes.

Recommendations

As already stated, a far larger and more extensive investigation may help to identify a link between hair metal concentration and POPs contents with mortality rates in the various boroughs investigated in Manchester, which it was hoped to uncover in this investigation. More samples per borough, and the through homogenisation of the collected hair samples, are other aspects requiring further attention. This type of investigation might also be repeated every five years to distinguish the longer-term trends over time. Trafford, as an outstanding borough, may require a separate investigation of its own, simply on the basis of the anomalies observed in this part of GM. The role of hair colouring in especially the POPs analyses may be a worthwhile peripheral idea to pursue. A larger variety of instrumentation, especially an ICP-MS for analysing the LOD and LOQ of the ICP-OES for certain metallic elements in some samples, would be a useful addition to any follow-up investigation.

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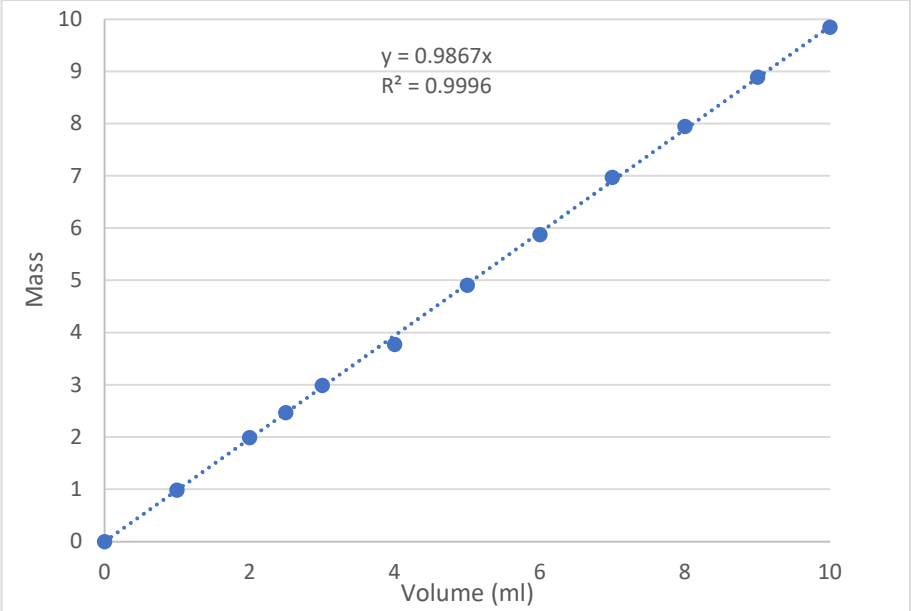
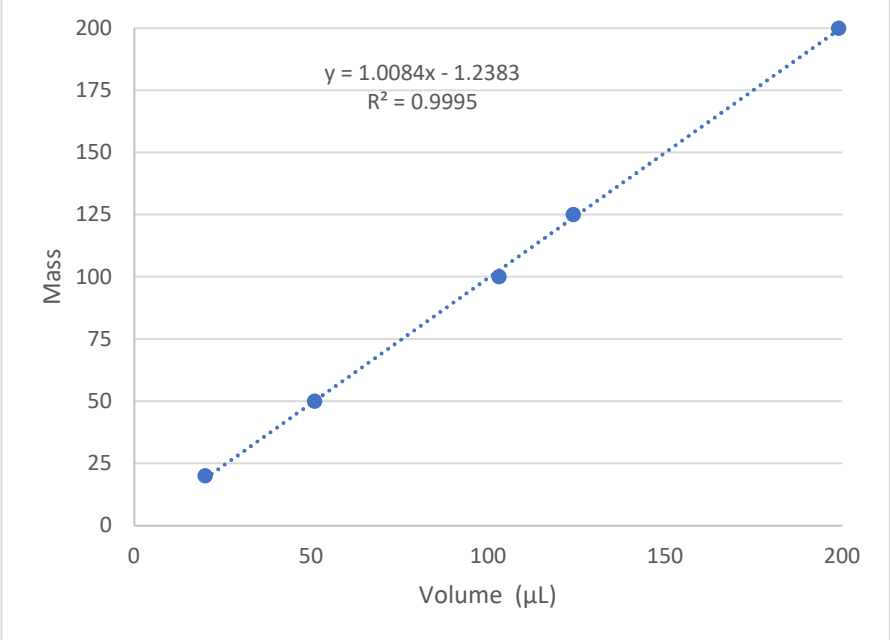
8. APPENDICES

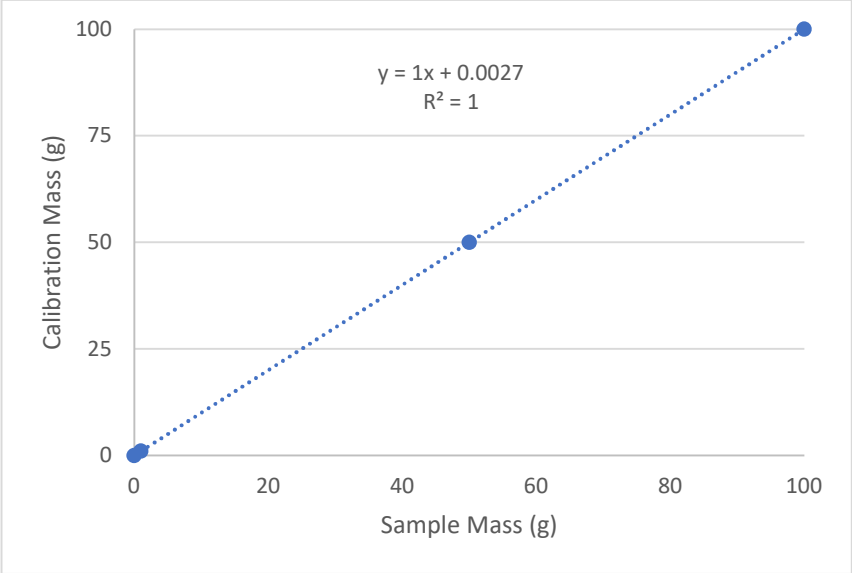
A: Calibrated of VWR Ergonomic High-Performance Eppendorf pipette (1-10ml)



B: Calibrated of VWR Ergonomic High-Performance Eppendorf pipette (20-200 µL)



C: Calibrated of a Sartorius model analytical balance scale



D: The Case Study Hair Sampling Details

Bolton (Central)

A1: Shop 1, Sample type A

A2: Shop 2, Sample type A

B1: Shop 1, Sample type B

B2: Shop 2, Sample type B

Bolton

All the samples collected from central Bolton on 31.05.2017, except A1 samples collected on 06/06/2017.

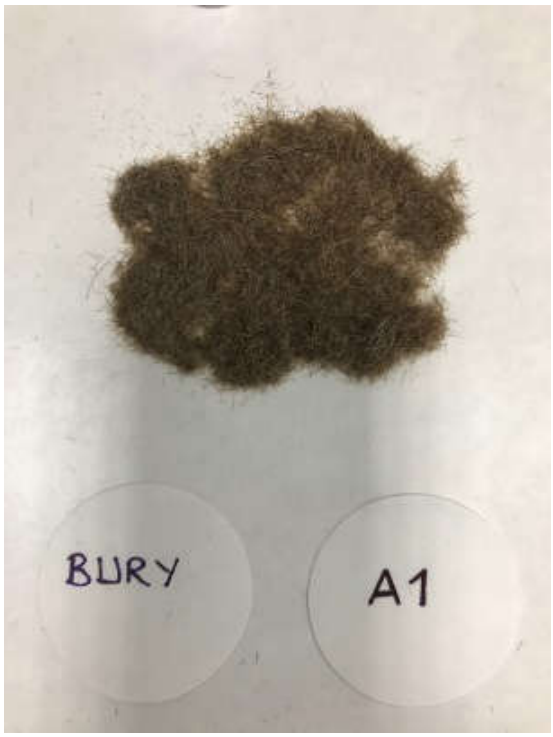
A1: Samples collected from four different hairdressers. There is no information provided for the presence of natural hair, but observation confirmed the existence of coloured hairs. 90% of the hair samples were brown: golden blond to light brown. The reminder was white, grey, light blond, hazelnut, burgundy and low amounts of black hair. The pictured samples were cleaned in the laboratory and ready for wash procedure. Total sample size estimated as 60 and age group as 3-60.

A2: Samples collected from two different hairdressers. 80 % of the hair samples were mainly light brown and grey colour. The blond, grey and some dark brown to black hairs also were present. The photographed samples were ready for the washing steps. Total size reported as 57 and the age group 5-80.

B1: Samples collected from multiple barbers. 80% of the hair was black and dark brown; also included less white, brown, grey and light brown hairs. The photographed samples were ready for wash steps. The total sample size noted as 63 and the age 4-65.

B2: The hair samples were very similar to B1 samples but interestingly these samples were shining. %95 of the sample was dark black, particularly comparison to whole area but were not oily. The nature of the black hair samples was not known. The pictured sample was ready for washing stage. Total sample size was estimated at 78 and age was between 10 to 70.

Bury (Central)



A1: Shop 1, Sample type A

A2: Shop 2, Sample type A

B1: Shop 1, Sample type B

B2: Shop 2, Sample type B

Bury

All the samples collected on 31 May 2017 from Bury central.

A1: 98% of the sample was light brown to blond. The reminder of the sample was white and grey. Black or dark brown hair samples could be seen only with a magnifying glass. There were no other colours observed. The pictured 1mm cut hair samples were ready for digestion or extraction method. Sample size reported as 65, and the age group as 6-60.

A2: Hair samples were very fine in comparison to all other Greater Manchester area samples. 80% of the sample was blond, around 15% was light brown. The rest was a combination of white and low levels of dark brown. The photographed sample was ready for wash stage. Age group was reported as 10-65 and sample size as 50.

B1: 90% of the hair was dark brown to black, the reminder consisted of blond, ginger, plum red, white and grey colours. Sample was ready for the wash step. Age group reported as 2-80, sample size as 54.

B2: 60% of the sample was grey and mid brown. The rest was a combination of white, blond, light brown and a low amount of black hairs. Sample was ready for wash step. Sample size noted as 64, age group 4-70.

Manchester (Central, Rusholme)

A1: Shop 1, Sample type A

A2: Shop 2, Sample type A

B1: Shop 1, Sample type B

B2: Shop 2, Sample type B

Manchester

A1 and A2 samples collected on 02/06/2017 from city centre, B1 and B2 samples collected on 21/06/2017 from Rusholme area.

A1: 80% of the samples were dark brown. Dark to light blond, ginger and black hair were also present. Pictured sample ready for wash step. Age group was 17-55 and sample size was 53.

A2: 90% of the hair samples were mostly light brown to blond. Medium brown, black and white was also seen. Pictured sample was ready for the wash step. Sample size reported as 55 and age 15-50.

B1: Out of the 36-sample site African Hair type was only present in this sample. Around 96% of the hair sample was dull black colour. However close examination of the samples indicated that white, grey, brown even burgundy colour hairs were also present. It was reported that most of the clients were from minority groups. Sample size noted as 70 and sample age variance given as 3-80.

B2: Hair samples were mainly black and dark brown. It was very difficult to distinguish colours. There were a few white hair strands in the sample. The pictured sample was ready for the wash step. The total sample reported as 65 and the age was 3-65. However, majority of the customers age was reported as between 20 to 55.

Oldham (Central)

A1: Shop 1, Sample type A

A2: Shop 2, Sample type A

B1: Shop 1, Sample type B

B2: Shop 2, Sample type B

Oldham

All the samples collected on 12 June 2017 from central town.

A1: Samples collected from three different hairdressers. One of them reported that 40% of the clients were male. The samples comprised of mainly grey and white hair, the presence of red, purple, blond, black and brown colours also noted. The pictured sample was ready for the washing step. Total sample size was 90. Age group was between 11-55.

A2: Samples collected from multiple hairdressers. 90% of the samples consisted of dark brown and hazel. Dark plum, gold, white-grey, light blond and black were present in the sample. Samples were very clean when collected, perhaps due to hairdressers washing after colouring and before cutting of the hair. Pictured samples were ready for the wash step. Sample size reported as 85, age group as 6-60.

B1: Samples collected from various barbers in town. The sample consist of approximately 50% white and grey, and 50% light brown. The presence of blond and black hair was hardly noticeable. Sample age noted as 1-80 and sample size as 95.

B2: Samples were collected from two different barbers. About 65% of the sample was grey colour. The rest was mostly light black and light brown. Very low amount plum red colour also seen when it was closely examined. The sample size was around 90, The age was 4-65.

Rochdale (Central)

A1: Shop 1, Sample type A

A2: Shop 2, Sample type A

B1: Shop 1, Sample type B

B2: Shop 2, Sample type B

Rochdale

A1and A2 collected on 31 May 2017, B1 and B2 samples collected on 02June 2017 from central.

A1: The samples were manly light such as white, light brown, light plum red, light blond. There were some black colours, but it was light and dull. Samples ready for the wash step. The sample age reported as 6-55 and sample size as 50.

A2: Samples were collected from various hairdressers. Samples consisted of mainly dark colours; black and brown. However, blond, all tones of brown, white, burgundy were present. The sample was ready for the wash process. Total sample size noted as 55 and the age group as 11-50.

B1: 98% of the sample was black. The rest consisted of brown, grey white and dark blond. The pictured samples were ready for the cutting process. The sample size reported as 80 and the age group as 1-50.

B2: Samples consist of mostly grey, white and ash colour. The rest was dark brown. The sample size reported as 75 and the age 5-60.

Salford (Central)

A1: Shop 1, Sample type A

A2: Shop 2, Sample type A

B1: Shop 1, Sample type B

B2: Shop 2, Sample type B

Salford

A and A2 samples collected on 31 May 2017, B1 and B2 samples collected on 02 June 2017.

A1: The collected samples were washed at the hairdresser before the hair clippings. Despite being washed previously, the samples re-washed according to methodology. The sample contained dark plum red, dark brown and black hairs only. There was no white, blond or grey hair. This was the only sample which included the long length of the hair. The sample size reported as 52 and the age group as 7-70.

A2: %95 of the sample was dark brown to black colour, the reminder consisted of light brown, burgundy, plum red and a low amount of white colours. The pictured sample had a final preparation and were ready for digestion or extraction step. Sample size reported as 55 and the age group as 8-55.

B1: The samples collected from multiple barbers. It has been reported that the barbers' clients were mostly from minority groups. Their clients were mainly Jewish. 90% of the sample was dark brown to black, although the rest of the hair appeared to be bright brown, white and grey colour. It was interesting see different hair shapes which called dreadlocks (as seen on image at the right). The sample size reported as 65 and the age group as 12- 60.

Dreadlocks found in **B1**

B2: The samples collected from four different shops. It has been reported that the barbers' clients were mostly from minority groups. Their clients were mainly Turkish or Kurdish. The pictured samples were ready for wash stage. %95 of the sample was black to dark brown, the reminder consisted of golden blond, white and grey. The pictured samples were ready for cleaning stage. Sample estimates as 70 and the age group reported as 4-80.

Stockport (Cheadle, Cheadle Hulme)

A1: Shop 1, Sample type A

A2: Shop 2, Sample type A

B1: Shop 1, Sample type B

B2: Shop 2, Sample type B

Stockport

All the samples collected between 18 to 30 of May, in 2017.

A1: The samples collected on 26 May 2017 from various hairdressers in Cheadle and Cheadle Hulme areas. %85 of the sample was light to dark blond colour. The rest was a burgundy, light brown, ash and black. However, black colour was the lowest amount in the sample. The photographed sample was ready for the wash stage. Sample size estimated as 80 and the age group reported as 6-80.

A2: The samples were collected on 25 May 2017 from various of the hairdressers in Cheadle village. The samples were very colorful and, mainly red and burgundy. Overall, the sample consisted of ginger, red, blond, plum red, dark brown, ash, black colours. The pictured sample was ready for the cleaning step. Sample size reported as 70 and the age group as 4- 80.

B1: The samples were collected from 4 different shops in Stockport between 18 May to 30 May in 2017. Sample size were reported as 130 and the age group as 4-70. The sample consisted of grey, white, light to dark brown and black colours and very low amount of blond.

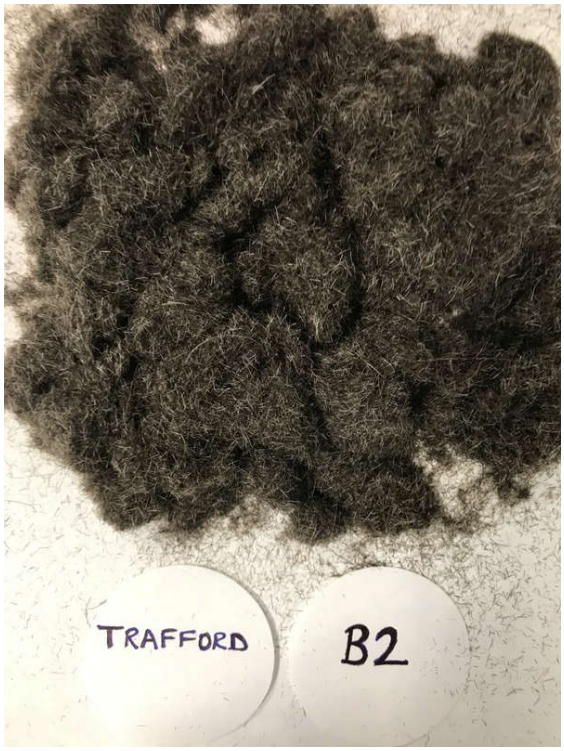
B2: Sample collected on 30 May 2017 from various barbers. Sample consisted of white, grey, black and dark brown colours. The pictured sample was ready for the wash sage. Sample size noted as 90 and sample age group as 6-85.

Trafford (Altringham, Sale)

A1: Shop 1, Sample type A

A2: Shop 2, Sample type A

B1: Shop 1, Sample type B



B2: Shop 2, Sample type B

Trafford

A1 and B2 samples collected on 3 May, B1 on 22 May, A2 on 23 May in 2017 in centre of Trafford.

A1: The sample consisted of brown and blond. There was no black colour. The photographed sample was ready for the cutting stage. Sample size reported as 52 and the age group as 10-55.

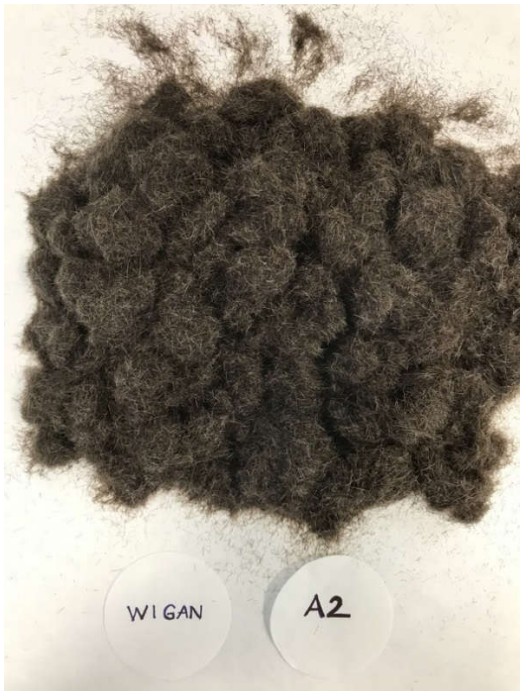
A2: %99 of the sample was ginger and bright brown. The reminder of the sample was blond. There was no black colour. The pictured sample was ready for the cutting stage. Sample size reported as 50 and the age group as 8-50.

B1: The sample consisted of grey, white and black colours. The pictured sample was ready for the digestion or extraction. Sample size reported as 110 and the age group as 9-65.

B2: The sample consisted of dark brown, grey, white and black colours. The photographed sample was ready for extraction or digestion. Sample size noted as 100 and the age group 8-70.

Wigan (Central)

A1: Shop 1, Sample type A



A2: Shop 2, Sample type A

B1: Shop 1, Sample type B

B2: Shop 2, Sample type B

Wigan

A1 and B2 samples collected on 18 May, A2 on 21 May and B2 on 11 May in 2017.

A1: the sample consisted of white, black and mostly dark brown. Perhaps after the Salford hair samples, A1 samples were the second most clean comparison to other samples in GM. Pictured hair sample was ready for homogenisation. The samples were counted individually at the hairdressers within their wish. Sample size reported as 65 and the age group as 4-85.

A2: The sample consisted of dark brown, white and grey and low amount of black. The pictured sample was ready for the digestion or extraction. Sample size estimated as 70 and the age group reported as 5-70.

B1: The sample consisted of mostly dull black and grey and less blond. The pictured sample was ready for the cleaning step. Sample size reported as 130 and the age group as 9-60.

B2:

%90 of the sample was dull black. The reminder of the sample was grey, white, plum red, dark brown and blond. The pictured sample was ready for the cleaning stage. Sample size estimated as 120, age as 5-70.

E: The letter for Proprietor/s: Hairdresser and/or Barber

Faculty of Science and Engineering

Graduate School

Proprietor/s: Hairdresser and / or Barber

2 May 2017

Address:

John Dalton Building

Chester Street

Manchester

M1 5GD

United Kingdom

E-mail

07452215@stu.mmu.ac.uk

To Whom It May Concern,

I am a postgraduate student at Manchester Metropolitan University. As part of my research project, I am analysing human hair cuttings for its chemical character. For this purpose, I am collecting hair samples from the Greater Manchester area. To enable me to have a good representation of the area, I have chosen different hairdressers / stylists / barbers from whom to collect samples. I hereby request permission to collect pooled hair cuttings from your establishment and would greatly appreciate your participation in this study by donating hair clippings.

The research will be conducted according to rules and regulations of the Manchester Metropolitan University and confidentiality will be protected under the Data Protection Act 1998. The data may be published in scientific journals, but the proprietors' business name and their identity will not be revealed. Furthermore, as the research will be conducted on mixed hair samples collected over a week, are only clippings and individual identity will not be discernible, there is no ethical concerns.

If you need further information, please do not hesitate to contact me by email. Thank you in advance.

Yours faithfully,

Hulya Kars

MSc Research Student

F: Statistical Analysis Details

1: Hair Treatment Optimisation

Cr mean level with unwashed male hair is 0,00054. Triton wash decreased level to 0,00012, while WAW decreased level to 0,00041 and AWA decreased level to 0,00040.

Cr mean level with unwashed composite hair is 0,00036. Triton wash decreased level to 0,00014, while WAW decreased level to 0,00018 and AWA decreased level to 0,00019.

Cr mean level with unwashed female hair is 0,00036. Triton wash decreased level to 0,00014, while WAW decreased level to 0,00018 and AWA decreased level to 0,00019.

Mn mean level with unwashed male hair is 0,00004. Triton wash decreased level to 0, while WAW decreased level to 0,00005 and AWA increased level to 0,00027.

Mn mean level with unwashed composite hair is 0,00033. Triton wash decreased level to 0,00028, while WAW increased level to 0,00051 and AWA increased level to 0,00053.

Mn mean level with unwashed female hair is 0,00009. Triton wash decreased level to 0,00002, while WAW decreased level to 0,00006 and AWA decreased level to 0,00004.

Ni mean level with unwashed male hair is 0,00089. Triton wash decreased level to 0,00006, while WAW and AWA decreased level to 0,00050.

Ni mean level with unwashed composite hair is 0,00085. Triton wash decreased level to 0,00056, while WAW decreased level to 0,00070 and AWA decreased level to 0,00066.

Ni mean level with unwashed female hair is 0,00097. Triton wash decreased level to 0,00095, while WAW decreased level to 0,00069 and AWA decreased level to 0,00073ppm.

Zn mean level with unwashed male hair is 0.20366 ppm. Triton wash decreased level to 0.18606ppm, while WAW decreased level to 0.19555ppm and AWA decreased level to 0.18153ppm.

Zn mean level with unwashed composite hair is 0.20374 ppm. Triton wash decreased level to 0.20284ppm, while WAW increased level to 0.22244ppm and AWA increased level to 0.22278ppm.

Zn mean level with unwashed female hair is 0.23723ppm. Triton wash decreased level to 0.23271ppm, while WAW decreased level to 0.22875ppm and AWA decreased level to 0.22015ppm.

Pb mean level with unwashed male hair is 0,00098. Triton wash decreased level to 0,00066 while WAW increased level to 0,00103 and AWA decreased level to 0,00081.

Pb mean level with unwashed composite hair is 0,00095. Triton wash decreased level to 0,00089, while WAW increased level to 0,00096 and AWA did not change the level.

Pb mean level with unwashed female hair is 0,00105. Triton wash decreased level to 0,00093, while WAW decreased level to 0,00095 and AWA decreased level to 0,00087.

One-way ANOVA conducted between elements vs Sample Types (female, male, composite)

For Cr, Mn, Ni and Zn there was a significant difference at the $p<0,05$ level for three conditions ([F(2, 116)=10.851, p=0.00] [F(2, 64)=26.599, p=0.00] [F(2, 114)=8.802, p=0.00] [F(2, 116)=33.796, p=0.00], respectively). Again, for Cr and Mn, Post hoc comparison using the Tamhane test applied and results indicated that the mean score for male (M= 0.000366, SD= 0.000200; M= 0.000272, SD= 0.000152, respectively) was higher and significantly different from female (M= 0.000219, SD= 0.000111; M= 0.000066, SD= 0.000046, respectively). However, using the same test for Ni, the results showed that the mean score for male (M=0.000510, SD= 0.000404) was lower and significantly different from female (M= 0.000832, SD= 0.000359). In addition, for Zn, Post hoc comparison using the Tukey test applied and results indicated that the mean score for male (M= 0.191700, SD= 0.025924) was lower and significantly different from female (M= 0.229633, SD= 0.014810).

One-way ANOVA conducted between Hair Treatment Types vs Elements

For Cr, Ni and Pb, there was a significant difference at the $p<0,05$ level for four conditions ([F(3, 115)=26.272, p=0.00] [F(3, 113)=6.263, p=0.001] [F(3, 115)=10.113, p=0.00], respectively) and Post hoc comparison using the Tukey test applied. The results for Cr indicated that the mean score for Triton wash hair (M=0.0001528, SD=0.00007620) was

lower and significantly different from unwashed (M= 0.000438, SD= 0.0001269), WAW (M= 0.000322, SD= 0.0001343) and AWA (M=. 0.000313, SD= 0.0001457). For Ni, the results were indicated that the mean score for unwashed hair (M= 0.0009026, SD= 0.0001849) was higher and significantly different from Triton wash hair (M= 0.0005429, SD= 0.0005531), WAW (M= 0.0006286, SD= 0.0002064) and AWA (M= 0.0006326, SD= 0.0003045). In contrast, for Pb, the mean score for AWA wash (M= 0.0008773, SD= 0.0001476) was lower and significantly different from unwashed (M= 0.0009936, SD= 0.0001289), and WAW (M= 0.00098, SD= 0.0001465).

F1a: Hair Treatment optimisation: Average values for the Statistical Analysis Results

	Cd				Cr				Mn			
	Unwashed	Triton	WAW	AWA	Unwashed	Triton	WAW	AWA	Unwashed	Triton	WAW	AWA
Male (Sample Type-B)	0.00000	0.00000	0.00000	0.00000	0.00054	0.00012	0.00041	0.00040	0.00004	0.00000	0.00005	0.00027
Composite	0.00002	0.00027	0.00002	0.00001	0.00036	0.00014	0.00018	0.00019	0.00033	0.00028	0.00051	0.00053
Female(Sample Type-A)	0.00007	0.00004	0.00004	0.00003	0.00036	0.00014	0.00018	0.00019	0.00009	0.00002	0.00006	0.00004
Min	0.00000	0.00000	0.00000	0.00000	0.00036	0.00012	0.00018	0.00019	0.00004	0.00000	0.00005	0.00004
Q1	0.00001	0.00002	0.00001	0.00001	0.00036	0.00013	0.00018	0.00019	0.00007	0.00001	0.00006	0.00016
Median	0.00003	0.00010	0.00002	0.00001	0.00042	0.00013	0.00026	0.00026	0.00015	0.00010	0.00021	0.00028
Q3	0.00005	0.00016	0.00003	0.00002	0.00045	0.00014	0.00030	0.00030	0.00021	0.00015	0.00029	0.00040
Max	0.00007	0.00027	0.00004	0.00003	0.00054	0.00014	0.00041	0.00040	0.00033	0.00028	0.00051	0.00053
Whiskers Top	0.00003	0.00012	0.00001	0.00001	0.00009	0.00000	0.00012	0.00011	0.00012	0.00013	0.00023	0.00013
Whiskers Bottom	0.00001	0.00002	0.00001	0.00001	0.00000	0.00001	0.00000	0.00000	0.00003	0.00001	0.00001	0.00012
	Ni				Pb				Zn			
Male	0.00089	0.00006	0.00050	0.00050	0.00098	0.00066	0.00103	0.00081	0.20366	0.18606	0.19555	0.18153
Composite	0.00085	0.00056	0.00070	0.00066	0.00095	0.00089	0.00096	0.00095	0.20374	0.20284	0.22244	0.22278
Female	0.00097	0.00095	0.00069	0.00073	0.00105	0.00093	0.00095	0.00087	0.23723	0.23271	0.22875	0.22015
Min	0.00085	0.00006	0.00050	0.00050	0.00095	0.00066	0.00095	0.00081	0.20366	0.18606	0.19555	0.18153
Q1	0.00087	0.00031	0.00060	0.00058	0.00097	0.00078	0.00096	0.00084	0.20370	0.19445	0.20900	0.20084
Median	0.00090	0.00052	0.00063	0.00063	0.00099	0.00083	0.00098	0.00088	0.21488	0.20720	0.21558	0.20815
Q3	0.00093	0.00076	0.00070	0.00070	0.00102	0.00091	0.00100	0.00091	0.22049	0.21778	0.22560	0.22147
Max	0.00097	0.00095	0.00070	0.00073	0.00105	0.00093	0.00103	0.00095	0.23723	0.23271	0.22875	0.22278
Whiskers Top	0.00004	0.00020	0.00001	0.00004	0.00004	0.00002	0.00004	0.00004	0.01675	0.01494	0.00316	0.00131
Whiskers Bottom	0.00002	0.00025	0.00010	0.00008	0.00001	0.00012	0.00001	0.00003	0.00004	0.00839	0.01345	0.01931

2: Case study

One-way ANOVA conducted between areas vs elements

For Al, there was a significant difference at the p<0,05 level for nine conditions [F(8, 63)=6.626, p=0.00]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Wigan (M=0.1825, SD=0.12563) is higher and significantly different from that for Bolton (M=0.1220, SD=0.11167) and Trafford (M=0.2144, SD=0.12563).

For Ca, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=8.497, p=0.00]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Trafford (M=2959.25, SD= 1365.06) is higher and significantly different from that for Bolton (M= 712.25, SD= 551.62), Manchester (M= 1180.33, SD= 814.34), Oldham (M=483.00, SD= 202.79), Rochdale (M= 977.41, SD= 713.20), Salford (M= 912.75, SD= 616.41) and Wigan (M= 1182.91, SD= 382.20). Also, the mean score for Wigan (M= 1182.91, SD= 382.20) is higher and significantly different from that for Oldham (M=483.00, SD= 202.79).

For Cr, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=3.864, p=0.01]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Rochdale (M=0.2525, SD=0.0245) is lower and significantly different from that for Stockport (M=0.3583, SD=0.0746).

For Cu, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=9.934, p=0.00]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Stockport (M= 15.99, SD=4.13) is lower and significantly different from that for Bolton (M= 10.27, SD= 2.14), Oldham (M= 10.67, SD= 1.42) and Salford (M= 10.39, SD= 2.08).

For Fe, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=2.119, p=0.041]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Bolton (M= 53.56, SD= 16.81) is higher and significantly different from that for Stockport (M= 29.42, SD= 13.24) and Trafford (M=

26.59, SD= 15.90). Also, the mean score for Wigan (M= 50.39, SD= 15.45) is higher and significantly different from that for Trafford (M= 26.59, SD= 15.90).

For K, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=3.997, p=0.00]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Wigan (M= 38.65, SD= 7.06) is lower and significantly different from that for Manchester (M= 122.07, SD= 60.27), Oldham (M= 210.16, SD= 114.42), Stockport (M=79.23, SD= 18.41) and Trafford (M= 84.95, SD= 21.52).

For Mg, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=3.810, p=0.01]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Oldham (M= 66.95, SD= 20.43) is lower and significantly different from that for Trafford (M= 195.83, SD=73.41) and Wigan (M= 121.73, SD= 32.91).

For Na, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=4.827, p=0.00]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Wigan (M= 88.6817, SD= 54.3665) is lower and significantly different from that for Manchester (M= 211.9167, SD= 72.7167), Bury (M= 324.7500, SD= 192.8334) and Oldham (M= 262.5000, SD= 119.6832).

For Ni, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=7.699, p=0.00]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Wigan (M= 1.1125, SD=0.43799) is higher and significantly different from that for Bury (M=0.5642, SD=0.17845), Oldham (M=0.4108, SD=0.10255), Rochdale (M=0.5050, SD=0.11229) and Salford (M=0.4275, SD=0.26728).

For S, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=5.384, p=0.00]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Manchester (M=35927.50, SD=924.29) is lower and significantly different from that for Salford (M= 37577.91, SD= 1125.25), Stockport (M=37746.16, SD=1307.06) and Wigan (M= 44118.00, SD= 6606.13). Also, the mean score for Oldham (M= 35713.00, SD=503.88) is lower and significantly different from that for

Salford (M= 37577.91, SD= 1125.25), Stockport (M=37746.16, SD=1307.06) and Wigan (M= 44118.00, SD= 6606.13).

For Zn, there was a significant difference at the p<0,05 level for nine conditions [F(8, 99)=7.632, p=0.00]. A post hoc comparison using the Tamhane test was applied and the results indicated that the mean score for Oldham (M= 161.83, SD= 28.61) is lower and significantly different from that for Salford (M= 210.75, SD= 34.38).

Independent Sample t-test for gender vs elements

For Al, there is a significant difference in the scores for the female (M=23.26, SD=16.36) and male (M=31.13, SD=16.36) conditions; t(92.92)=-2.93, p=0.00.

For As, there is a significant difference in the scores for the female (M=0.37, SD=0.37) and male (M=0.74, SD=0.69) conditions; t(68.46)=-2.88, p=0.014.

For Ca, there is a significant difference in the scores for the female (M=1797.18, SD=1005.32) and male (M=741.29, SD=846.21) conditions; t(106)=5.90, p=0.00.

For Fe, there is a significant difference in the scores for the female (M=25.56, SD=21.26) and male (M=51, SD=25.70) conditions; t(106)=-4.72, p=0.00.

For Mg, there is a significant difference in the scores for the female (M=216.29, SD=125) and male (M=80.27, SD=49.30) conditions; t(69.10)=7.43, p=0.00.

For Mn, there is a significant difference in the scores for the female (M=0.74, SD=0.58) and male (M=1.17, SD=1.06) conditions; t(82.24)=-2.62, p=0.10.

For P, there is a significant difference in the scores for the female (M=132.9, SD=15.2) and male (M=168.9, SD=38.1) conditions; t(106)=-6.44, p=0.00.

Independent Sample t-test for Bolton1 & Bolton2 vs elements

For Na, there is a significant difference in the scores for the Bolton1 (M=243.33, SD=10.09) and Bolton2 (M=60.41, SD=16.85) conditions; t(8.17)=22.06, p=0.00.

For Zn, there is a significant difference in the scores for the Bolton1 (M=157, SD=39.1) and Bolton2 (M=228.66, SD=61.86) conditions; t(8.47)=-2.39, p=0.042.

Independent Sample t-test for Bury1 & Bury2 vs elements

For As, there is a significant difference in the scores for the Bury1 (M=0.33, SD=0.20) and Bury2 (M=1.32, SD=0.14) conditions; t(4)=-12.156, p=0.00.

For Zn, there is a significant difference in the scores for the Bury1 (M=189.33, SD=30.31) and Bury2 (M=141.66, SD=16.95) conditions; t(7.8)=3.36, p=0.010.

Independent Sample t-test for Manchester1 & Manchester2 vs elements

For As, there is a significant difference in the scores for the Manchester1 (M=0.27, SD=0.24) and Manchester2 (M=0.90, SD=0.40) conditions; t(8.14)=-3.22, p=0.012.

For Na, there is a significant difference in the scores for the Manchester1 (M=266.3, SD=60.3) and Manchester2 (M=157.5, SD=29.7) conditions; t(7.3)=3.96, p=0.005.

For K, there is a significant difference in the scores for the Manchester1 (M=157.8, SD=50.2) and Manchester2 (M=86.31, SD=48.98) conditions; t(10)=2.49, p=0.032.

For Pb, there is a significant difference in the scores for the Manchester1 (M=0.77, SD=0.10) and Manchester2 (M=0.94, SD=0.13) conditions; t(10)=-2.46, p=0.033.

For Zn, there is a significant difference in the scores for the Manchester1 (M=152, SD=15.23) and Manchester2 (M=382.3, SD=201.3) conditions; t(5.05=-2.79, p=0.038.

Independent Sample t-test for Oldham1 & Oldham2 vs elements

For Cr, there is a significant difference in the scores for the Oldham1 (M=0.27, SD=0.38) and Oldham2 (M=0.32, SD=0.42) conditions; t(10)=-2.34, p=0.041.

For Cu, there is a significant difference in the scores for the Oldham1 (M=9.74, SD=1.01) and Oldham2 (M=11.61, SD=1.14) conditions; t(10)=-3.001, p=0.013.

For Ni, there is a significant difference in the scores for the Oldham1 (M=0.33, SD=0.38) and Oldham2 (M=0.48, SD=0.08) conditions; t(10)=-3.84, p=0.003.

For Pb, there is a significant difference in the scores for the Oldham1 (M=0.84, SD=0.17) and Oldham2 (M=3.04, SD=0.90) conditions; t(5.39)=-5.86, p=0.002.

Independent Sample t-test for Rochdale1 & Rochdale2 vs elements

For As, there is a significant difference in the scores for the Rochdale1 (M=0.78, SD=0.34) and Rochdale2 (M=2.66, SD=0.35) conditions; t(7)=-7.55, p=0.00.

For K, there is a significant difference in the scores for the Rochdale1 (M=299, SD=210.83) and Rochdale2 (M=41.35, SD=18.50) conditions; t(5.07)=2.98, p=0.030.

For Na, there is a significant difference in the scores for the Rochdale1 (M=276.16, SD=156.99) and Rochdale2 (M=53.81, SD=7.68) conditions; t(5.02)=3.46, p=0.018.

For Pb, there is a significant difference in the scores for the Rochdale1 (M=1.46, SD=0.14) and Rochdale2 (M=0.72, SD=0.27) conditions; t(7.56)=5.87, p=0.00.

For Zn, there is a significant difference in the scores for the Rochdale1 (M=361.66, SD=212.61) and Rochdale2 (M=152, SD=4.28) conditions; t(5.004)=2.41, p=0.060.

Independent Sample t-test for Salford1 & Salford2 vs elements

For As, there is a significant difference in the scores for the Salford1 (M=0.30, SD=0.03) and Salford2 (M=0.05, SD=0.03) conditions; t(4)=9.28, p=0.001.

For Pb, there is a significant difference in the scores for the Salford1 (M=1.01, SD=0.11) and Salford2 (M=0.71, SD=0.23) conditions; t(7.3)=2.80, p=0.025.

Independent Sample t-test for Stockport1 & Stockport 2 vs elements

For As, there is a significant difference in the scores for the Stockport1 (M=1.68, SD=0.08) and Stockport2 (M=0.18, SD=0.00) conditions; t(2)=15.67, p=0.004.

For Cr, there is a significant difference in the scores for the Stockport1 (M=0.40, SD=0.31) and Stockport2 (M=0.31, SD=0.07) conditions; t(10)=2.26, p=0.047.

Independent Sample t-test for Trafford1 & Trafford 2 vs elements

For Cu, there is a significant difference in the scores for the Trafford1 (M=30.68, SD=9.51) and Trafford2 (M=110.01, SD=61.84) conditions; t(5.23)=-3.10, p=0.025.

For K, there is a significant difference in the scores for the Trafford1 (M=71.23, SD=10.08) and Trafford2 (M=98.68, SD=21.57) conditions; t(10)=-2.83, p=0.018.

For Mg, there is a significant difference in the scores for the Trafford1 (M=149.33, SD=37.50) and Trafford2 (M=242.33, SD=72.54) conditions; t(10)=-2.79, p=0.019.

For Mn, there is a significant difference in the scores for the Trafford1 (M=0.99, SD=0.37) and Trafford2 (M=3.66, SD=1.32) conditions; t(5.78)=-4.73, p=0.004.

For Na, there is a significant difference in the scores for the Trafford1 (M=244.50, SD=62.37) and Trafford2 (M=1.66, SD=0.91) conditions; t(5.002)=9.53, p=0.00.

Independent Sample t-test for Wigan1 & Wigan2 vs elements

For Al, there is a significant difference in the scores for the Wigan1 (M=26.93, SD=2.98) and Wigan2 (M=31.70, SD=3.00) conditions; t(10)=-2.75, p=0.020.

For Ca, there is a significant difference in the scores for the Wigan1 (M=1519.16, SD=59.42) and Wigan2 (M=846.66, SD=215.63) conditions; t(5.75)=7.36, p=0.00.

For Cr, there is a significant difference in the scores for the Wigan1 (M=0.33, SD=0.08) and Wigan2 (M=0.84, SD=0.13) conditions; t(10)=-7.925, p=0.00.

For Fe, there is a significant difference in the scores for the Wigan1 (M=38.18, SD=12.69) and Wigan2 (M=62.60, SD=2.55) conditions; t(10)=-4.61, p=0.001.

For K, there is a significant difference in the scores for the Wigan1 (M=31.93, SD=0.96) and Wigan2 (M=45.38, SD=0.65) conditions; t(8.81)=-28.28, p=0.00.

For Mn, there is a significant difference in the scores for the Wigan1 (M=1.21, SD=0.16) and Wigan2 (M=2.36, SD=1.02) conditions; t(5.2)=-2.71, p=0.040.

For Ni, there is a significant difference in the scores for the Wigan1 (M=0.78, SD=0.09) and Wigan2 (M=1.43, SD=0.39) conditions; t(5.57)=-3.90, p=0.009.

G: Metal and POP Profile Final Concentrations

Metal Profile Final Concentration, mg kg^{-1}, female (A) and male (B)

Suburban Area ---------- A - Sample Type	Trace elements (mg kg^{-1})						Minor elements (mg kg^{-1})								Major elements (% (m/m))	
	As	Cd	Cr	Mn	Ni	Pb	Zn	Al	Cu	Fe	K	Mg	Na	P	Ca	S
Bolton	0.18	-	0.25	0.87	0.70	0.83	147	35.7	12.0	42.7	45.0	140	152	146	0.10	3.64
Bury	-	-	0.25	0.52	0.66	0.40	144	13.9	22.1	19.1	109	439	435	115	0.25	3.63
Manchester	0.67	0.02	0.76	0.56	1.00	0.87	352	28.6	16.8	52.6	76.7	198	226	124	0.20	3.58
Oldham	0.12	-	0.30	0.54	0.40	1.46	164	26.7	11.6	30.8	143	84.9	190	149	0.07	3.56
Rochdale	0.47	-	0.25	0.41	0.60	0.92	352	12.8	14.0	19.4	258	195	240	135	0.16	3.58
Salford	0.07	0.04	0.29	0.35	0.41	0.93	241	9.72	11.3	15.2	13.5	218	52.3	132	0.14	3.71
Stockport	-	0.02	0.32	0.37	1.88	0.54	128	26.8	19.8	16.9	96.0	359	254	124	0.27	3.65
Trafford	0.17	0.05	0.32	1.74	1.33	24.6	489	25.6	65.1	17.7	79.8	207	151	126	0.33	4.46
Wigan	0.55	0.01	0.62	1.33	1.21	7.01	238	29.7	13.4	51.6	39.0	106	123	147	0.11	4.04
B - Sample Type	**As**	**Cd**	**Cr**	**Mn**	**Ni**	**Pb**	**Zn**	**Al**	**Cu**	**Fe**	**K**	**Mg**	**Na**	**P**	**Ca**	**S**
Bolton	0.04	-	0.36	0.90	0.44	0.99	239	35.7	8.58	64.4	150	57.3	158	183	0.05	3.59
Bury	0.83	-	0.25	0.52	0.66	0.40	144	13.9	22.1	19.1	109	439	435	115	0.25	3.63
Manchester	0.67	0.02	0.76	0.56	1.00	0.87	352	28.6	16.8	52.6	76.7	198	226	124	0.20	3.58
Oldham	0.60	-	0.30	0.66	0.42	2.42	160	23.5	9.74	42.2	278	49.0	335	157	0.03	3.59
Rochdale	1.88	-	0.26	0.69	0.41	1.28	162	23.6	9.27	46.8	82.4	67.8	90.1	177	0.03	3.66
Salford	0.16	0.01	0.37	0.90	0.44	0.80	181	56.0	9.45	78.1	155	48.7	196	165	0.04	3.80
Stockport	0.94	0.02	0.33	0.55	1.59	0.78	162	29.0	16.8	28.2	78.0	196	164	151	0.15	3.76
Trafford	0.13	0.07	0.49	2.93	1.86	58.2	873	30.4	75.6	35.5	90.2	184	94.7	198	0.27	6.43
Wigan	0.41	0.02	0.57	2.25	1.01	16.3	305	30.2	27.5	49.2	38.3	137	54.1	158	0.13	4.78

PCBs concentration, ng g^{-1}, female and male

A-Sample Types (Female)	PCB52	PCB118	PCB153	PCB209	B-Sample Types (Male)	PCB52	PCB118	PCB153	PCB209
Bolton	230	855	48	73	Bolton	274	624	83	74
Bury	393	3484	135	59	Bury	271	142	81	72
Manchester	186	316	84	65	Manchester	237	97	66	68
Oldham	213	646	60	60	Oldham	236	276	64	63
Rochdale	222	377	90	56	Rochdale	216	273	54	58
Salford	285	95	81	60	Salford	222	228	61	-
Stockport	203	250	94	61	Stockport	285	519	83	-
Trafford	137	441	189	63	Trafford	222	98	47	58
Wigan	143	369	81	59	Wigan	287	632	59	60

PAHs concentration, ng g^{-1}, female samples

A-Sample Types	2-Methylnaphthale	Acenaphthylene	Benzo(b) fluoranthene	Benzo(k) fluoranthene	Benzo(a) pyrene	Dibenza(a,h) anthracene	Naphthalene	Acenaphthene	Anthracene
Bolton	169	456	108	200	62.7	70.7	3432	17264	19108
Bury	222	1318	-	1506	41.8	151	5035	29713	1763
Manchester	181	493	318	701	114	76.0	16681	19812	1670
Oldham	124	935	272	228	75.3	43.0	3032	14525	1501
Rochdale	140	318	141	145	145	15.9	1557	6413	460
Salford	224	650	269	103	97.6	50.5	3049	14926	1876
Stockport	157	527	203	310	113	29.0	2361	54722	734
Trafford	249	1143	499	1004	117	40.2	11817	21364	6308
Wigan	185	684	71.1	157	96.3	29.9	7784	15763	8152

PAHs concentration , ng g^{-1}, male samples

B-Sample Types	Naphthalene	2-Methylnaphthale	Acenaphthylene	Acenaphthene	Fluorene	Anthracene	Benzo(b) fluoranthene	Benzo(k) fluoranthene	Benzo(a) pyrene	Dibenza(a,h) anthracene
Bolton	11757	151	488	18933	633	4709	254	191	93	74
Bury	9932	179	725	8727	494	1727	52	140	103	100
Manchester	15977	201	5343	18492	283	1800	129	213	53	61
Oldham	3620	185	753	17406	303	1729	109	210	62	48
Rochdale	9576	170	534	16070	616	2325	140	3379	53	38
Salford	3112	163	623	15862	457	1600	168	238	27	42
Stockport	6963	330	1374	29634	484	2472	49	122	109	80
Trafford	2022	146	601	9618	392	1106	73	33	55	30
Wigan	3755	173	913	18565	523	2279	-	92	84	33